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<p>(21) International Application Number: PCT/US96/10229</p> <p>(22) International Filing Date: 7 June 1996 (07.06.96)</p> <p>(30) Priority Data: 08/477,776 7 June 1995 (07.06.95) US</p> <p>(71) Applicant: SLOAN-KETTERING INSTITUTE FOR CANCER RESEARCH [US/US]; 1275 York Avenue, New York, NY 10021 (US).</p> <p>(72) Inventors: DANISHEFSKY, Samuel, J.; 22 Brayton Street, Englewood, NJ 07631 (US). ROBERGE, Jacques; Apartment 3A, 66 Linden Lane, Princeton, NJ 08540 (US). BEEBE, Xenia; Apartment 140, 1233 York Avenue, New York, NY 10021 (US).</p> <p>(74) Agent: WHITE, John, P.; Cooper & Dunham L.L.P., 1185 Avenue of the Americas, New York, NY 10036 (US).</p>		<p>(81) Designated States: AU, CA, JP, MX, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).</p> <p>Published <i>With international search report.</i></p>
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<div style="text-align: right; margin-top: -40px;"> <p>(I)</p> </div>		
<p>(57) Abstract</p> <p>The present invention provides a process for synthesizing a glycopeptide having structure (I), wherein R₁, R₂, R₃, R₄, R₅, R₆, R₇ and R₈ are each independently H, OH, OR¹, NH₂, NHCOR¹, F, CH₂OH, CH₂OR¹, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R¹ is H, CHO, CO₂R¹¹, a linear or branched chain alkyl, arylalkyl or aryl group, or an oligosaccharide moiety, etc., wherein R₉ is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein R₁₀ is a substituted or unsubstituted linear or branched chain acyl, arylacyl or aroyl group; wherein R₁₁[X] represents X amino acid side-chains, and denotes position from the N-terminus, and R₁₃[Y] represents Y amino acid side-chains, and denotes position from the C-terminus, wherein a, b, c, h, i, j, r, s and t are each independently an integer between about 0 and about 3; wherein m and n are each independently an integer between about 0 and about 5; and wherein x, x'(N), y and y'(N) are each independently an integer between about 0 and about 25; such glycopeptides being useful as a vaccine for inducing antibodies to human breast cancer cells in an adjuvant therapy therefor.</p>		

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SYNTHESIS OF ASPARAGINE-LINKED GLYCOPEPTIDES
ON A POLYMERIC SOLID SUPPORT

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This application is a continuation-in-part of U.S. Serial No. 08/430,355, filed April 28, 1995, which was a continuation-in-part of U.S. Serial No. 08/213,053, filed March 15, 1994, the contents of which are hereby incorporated by reference into this application.

This invention was made with government support under grants GM-15240-02, GM-16291-01, and AI-16943 from the National Institutes of Health. Accordingly, the U.S. Government has certain rights in the invention.

Background of the Invention

Throughout this application, citations for various publications are provided within parentheses in the text. The disclosures of these publications are hereby incorporated in their entirety by reference into this application in order to more fully describe the state of the art to which this invention pertains.

The function of carbohydrates as structural materials and as energy storage units in biological systems is well recognized. By contrast, the role of carbohydrates as signaling molecules in the context of biological processes has only recently been appreciated. (M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, J.C. Paulson, *Science*, 1990, 250, 1130; M.J. Polley, M.L. Phillips, E. Wagner, E. Nudelman, A.K. Singhal, S. Hakomori, J.C. Paulson, *Proc. Natl. Acad. Sci. USA*, 1991, 88, 6224; T. Taki, Y. Hirabayashi, H. Ishikawa, S. Kon, Y. Tanaka, M. Matsumoto, *J. Biol. Chem.*, 1986, 261, 3075; Y. Hirabayashi, A. Hyogo, T. Nakao, K. Tsuchiya, Y. Suzuki, M. Matsumoto, K. Kon, S. Ando, *ibid.*, 1990, 265, 8144; O. Hindsgaul, T. Norberg,

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J. Le Pendu, R. U. Lemieux, *Carbohydr. Res.*, 1982, 109, 109; U. Spohr, R.U. Lemieux, *ibid.*, 1988, 174, 211)

The elucidation of the scope of carbohydrate involvement in mediating cellular interaction is an important area of inquiry in contemporary biomedical research. The carbohydrate molecules, carrying detailed structural information, tend to exist as glycoconjugates (cf. glycoproteins and glycolipids) rather than as free entities. Given the complexities often associated with isolating the conjugates in homogeneous form and the difficulties in retrieving intact carbohydrates from these naturally occurring conjugates, the applicability of synthetic approaches is apparent. (For recent reviews of glycosylation see: Paulsen, H., *Angew. Chemie Int. Ed. Engl.*, 1982, 21, 155; Schmidt, R.R., *Angew. Chemie Int. Ed. Engl.*, 1986, 25, 212; Schmidt, R.R., *Comprehensive Organic Synthesis*, Vol. 6, Chapter 1(2), Pergamon Press, Oxford, 1991; Schmidt, R.R., *Carbohydrates, Synthetic Methods and Applications in Medicinal Chemistry*, Part I, Chapter 4, VCH Publishers, Weinheim, New York, 1992. For the use of glycals as glycosyl donors in glycoside synthesis, see Lemieux, R.U., *Can. J. Chem.*, 1964, 42, 1417; Lemieux, R.U., Fraiser-Reid, B., *Can. J. Chem.*, 1965, 43, 1460; Lemieux, R.U., Morgan, A.R., *Can. J. Chem.*, 1965, 43, 2190; Thiem, J., Karl, H., Schwentner, J., *Synthesis*, 1978, 696; Thiem, J., Ossowski, P., *Carbohydr. Chem.*, 1984, 3, 287; Thiem, J., Prahst, A., Wendt, T. *Liebigs Ann. Chem.*, 1986, 1044; Thiem, J. in *Trends in Synthetic Carbohydrate Chemistry*, Horton, D., Hawkins, L.D., McGarvey, G.L., eds., ACS Symposium Series No. 386, American Chemical Society, Washington, D.C., 1989, Chapter 8.)

The carbohydrate domains of the blood group substances contained in both glycoproteins and glycolipids are distributed in erythrocytes, epithelial cells and various secretions. The early focus on these systems centered on

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their central role in determining blood group specificities. (R.R. Race and R. Sanger, *Blood Groups in Man*, 6th ed., Blackwell, Oxford, 1975) However, it is recognized that such determinants are broadly implicated in cell adhesion and binding phenomena. (For example, see M.L. Phillips, E. Nudelman, F.C.A. Gaeta, M. Perez, A.K. Singhal, S. Hakomori, J.C. Paulson, *Science*, 1990, 250, 1130.) Moreover, ensembles related to the blood group substances in conjugated form are encountered as markers for the onset of various tumors. (K.O. Lloyd, *Am. J. Clinical Path.*, 1987, 87, 129; K.O. Lloyd, *Cancer Biol.*, 1991, 2, 421) Carbohydrate-based tumor antigenic factors have applications at the diagnostic level, as resources in drug delivery or ideally in immunotherapy. (Toyokuni, T., Dean, B., Cai, S., Boivin, D., Hakomori, S., and Singhal, A.K., *J. Am. Chem Soc.*, 1994, 116, 395; Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Paardoll, D., Mulligan, R., *Proc. Natl. Acad. Sci. USA*, 1993, 90, 3539; Tao, M-H., Levy, R., *Nature*, 1993, 362, 755; Boon, T., *Int. J. Cancer*, 1993, 54, 177; Livingston, P.O., *Curr. Opin. Immunol.*, 1992, 4, 624; Hakomori, S., *Annu. Rev. Immunol.*, 1984, 2, 103; K. Shigeta, et al., *J. Biol. Chem.*, 1987, 262, 1358)

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The present invention provides new strategies and protocols for glycopeptide synthesis. The object is to simplify such constructions such that relatively complex domains can be assembled with high stereospecificity. Major advances in glycoconjugate synthesis require the attainment of a high degree of convergence and relief from the burdens associated with the manipulation of blocking groups. Another requirement is that of delivering the carbohydrate determinant with appropriate provision for conjugation to carrier proteins or lipids. (Bernstein, M.A., and Hall, L.D., *Carbohydr. Res.*, 1980, 78, C1; Lemieux, R.U., *Chem. Soc. Rev.*, 1978, 7, 423;

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R.U. Lemieux, et al., *J. Am. Chem. Soc.*, 1975, 97, 4076)
This is a critical condition if the synthetically derived
carbohydrates are to be incorporated into carriers
suitable for biological application.

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Antigens which are selective or ideally specific for
cancer cells could prove useful in fostering active
immunity. (Hakomori, S., *Cancer Res.*, 1985, 45, 2405-
2414; Feizi, T., *Cancer Surveys*, 1985, 4, 245-269) Novel
10 carbohydrate patterns are often presented by transformed
cells as either cell surface glycoproteins or as
membrane-anchored glycolipids. In principle, well chosen
synthetic glycoconjugates which stimulate antibody
production could confer active immunity against cancers
15 which present equivalent structure types on their cell
surfaces. (Dennis, J., *Oxford Glycosystems Glyconews*
Second, 1992; Lloyd, K. O., in *Specific Immunotherapy of*
Cancer with Vaccines, 1993, New York Academy of Sciences
pp. 50-58) Chances for successful therapy improve with
20 increasing restriction of the antigen to the target cell.
A glycosphingolipid was isolated by Hakomori and
collaborators from the breast cancer cell line MCF-7 and
immunocharacterized by monoclonal antibody MBrl.
(Bremer, E. G., et al., *J. Biol. Chem.*, 1984, 259, 14773-
25 14777; Menard, S., et al., *Cancer Res.*, 1983, 43, 1295-
1300) The novel glycosphingolipid structure 1b (Figure
8) was proposed for this breast tumor-associated antigen
on the basis of methylation and enzymatic degradation
protocols. A ¹H NMR spectrum consistent with but not
30 definitive for the proposed structure was obtained from
trace amounts of isolated antigen. While individual
sectors of the proposed structure were not unknown, the
full structure was first described based on studies on
the breast cancer line. It should be noted that MBrl
35 also binds to normal human mammary gland tissue and
ovarian cancer cell lines. Therefore, 1b as a total
entity is likely not restricted to the transformed breast

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cells. Alternatively, smaller subsections of 1b are adequate for antibody recognition and binding. (The synthesis of the DEF fragment of 1b has been reported, and has been shown to bind to MBr1: Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *Helv. Chim. Acta*, 1994, 77, 509-514.)

The compounds prepared by processes described herein are antigens useful in adjuvant therapies as vaccines capable of inducing MBr1 antibodies immunoreactive with human breast tumor cells. Such adjuvant therapies have potential to reduce the rate of recurrence of breast cancer and increase survival rates after surgery. Clinical trials on 122 patients surgically treated for AJCC stage III melanoma who were treated with vaccines prepared from melanoma differentiation antigen GM2 (another tumor antigen which like MBr1 is a cell surface carbohydrate) demonstrated in patients lacking the antibody prior to immunization, a highly significant increase in disease-free interval (P.O. Livingston, et al., *J. Clin Oncol.*, 1994, 12, 1036).

The present invention provides a method of synthesizing 1b in quantity as well as artificial protein-conjugates of the oligosaccharide which might be more immunogenic than the smaller glycolipid. The antigen contains a novel array of features including the α -linkage between the B and the C entities, as well as the β -linked ring D gal-NAC residue. (For the synthesis of a related structure (SSEA-3) which lacks the fucose residue see: Nunomura, S.; Ogawa, T., *Tetrahedron Lett.*, 1988, 29, 5681-5684.) The present invention provides (i) a total synthesis of 1b, (ii) rigorous proof that the Hakomori antigen does, in fact, correspond to 1b and (iii) the synthesis of a bioconjugatable version of 1b. The conciseness of the synthesis reflects the efficiency of glycal assembly methods augmented by a powerful method

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for sulfonamidoglycosylation (see, e.g., the transformation of 14b-15b, Figure 10).

The surge of interest in glycoproteins (M. J. McPherson, P. Quirke, F. R. Taylor, Eds., *PCR A Practical Approach*, 1994, Oxford University Press, Oxford; G. M. Blackburn and M. J. Gait, Eds., *Nucleic Acids in Chemistry and Biology*, 1990, Oxford University Press, Oxford; A. M. Bray, A. G. Jhingran, R. M. Valerio, N.J. Maeji, *J. Org. Chem.* 1994, 59, 2197; G. Jung and A. G. Beck-Sickinger, *Angew. Chem. Int. Ed. Engl.* 1992, 31, 367; M. A. Gallop, R. W. Barrett, W. J. Dower, S. P. A. Fodor, E. M. Gordon, *J. Med. Chem.*, 1994, 37, 1233; H. P. Nestler, P. A. Bartlett, W. C. Still, *J. Org. Chem.*, 1994, 59, 4723; M. Meldal, *Curr. Opin. Struct. Biol.*, 1994, 4, 710; T. Feizi and D. Bundle, *Curr. Opin. Struct. Biol.*, 1994, 4, 673) arises from heightened awareness of their importance in diverse biochemical processes including cell growth regulation, binding of pathogens to cells (O.P. Bahl, in *Glycoconjugates: Composition, structure, and function*, H. J. Allen, E. C. Kisailus, Eds., 1992, Marcel Dekker, Inc., New York, p. 1) intercellular communication and metastasis (A. Kobata, *Acc. Chem. Res.*, 1993, 26, 319). Glycoproteins serve as cell differentiation markers and assist in protein folding and transport, possibly by providing protection against proteolysis (G. Opdenakker, P. M. Rudd, C. P. Ponting, R. A. Dwek, *FASEB J.*, 1993, 7, 1330). Improved isolation techniques and structural elucidation methods (A. Dell and K.-H. Khoo, *Curr. Opin. Struct. Biol.*, 1993, 3, 687) have revealed high levels of microheterogeneity in naturally-produced glycoproteins (R. A. Dwek, C. J. Edge, D. J. Harvey, M. R. Wormald, R. B. Parekh, *Annu. Rev. Biochem.*, 1993, 62, 65). Single eukaryotic cell lines often produce many glycoforms of any given protein sequence. For instance, erythropoietin (EPO), a clinically useful red blood cell stimulant against anemia, is glycosylated by more than 13 known

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types of oligosaccharide chains when expressed in Chinese hamster ovary cells (CHO) (Y. C. Lee and R. T. Lee, Eds., *Neoglycoconjugates: Preparation and Applications*, 1994, Academic Press, London). The efficacy of erythropoietin is heavily dependent on the type and extent of glycosylation (E. Watson, A. Bhide, H. van Halbeek, *Glycobiology*, 1994, 4, 227).

Elucidation of the biological relevance of particular glycoprotein oligosaccharide chains requires access to pure entities, heretofore obtained by isolation. Glycoprotein heterogeneity renders this process particularly labor-intensive. However, particular cell lines can be selected to produce more homogeneous glycoproteins for structure-activity studies (M. A. Lehrman and Z. Yucheng, U.S. Patent No. 5,272,070 (1993)). However, the problem of isolation from natural sources remains difficult.

Receptors normally recognize only a small fraction of a given macromolecular glycoconjugate. Consequently, synthesis of smaller but well-defined putative glycopeptide ligands could emerge as competitive with isolation as a source of critical structural information (Y. C. Lee and R. T. Lee, Eds., *Neoglycoconjugates: Preparation and Applications*, 1994, Academic Press, London). Prior to the subject invention, methods of glycopeptide synthesis pioneered by Kunz and others allowed synthetic access to homogenous target systems both in solution and solid phase (M. Meldal, *Curr. Opin. Struct. Biol.*, 1994, 4, 710 ; M. Meldal, in *Neoglycoconjugates: Preparation and Applications*, 1994, Y. C. Lee, R. T. Lee, Eds., Academic Press, London; S. J. Danishefsky and J. Y. Roberge, in *Glycopeptides and related compounds: Chemical synthesis, analysis and applications*, 1995, D. G. Large, C. D. Warren, Eds., Marcel Dekker, New York; S. T. Cohen-Anisfeld and P. T.

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Lansbury, Jr., *J. Am. Chem. Soc.*, 1993, 115, 10531; S. T. Anisfeld and P. T. Lansbury Jr., *J. Org. Chem.*, 1990, 55, 5560; D. Vetter, D. Tumelty, S. K. Singh, M. A. Gallop, *Angew. Chem. Int. Ed. Engl.*, 1995, 34, 60-63). Cohen-
5 Anisfeld and Lansbury disclosed a convergent solution-based coupling of selected already available saccharides with peptides (S. T. Cohen-Anisfeld and P. T. Lansbury, Jr., *J. Am. Chem. Soc.*, ibid.).

10 In the subject invention, the terminal glycal on the polymeric solid phase is linked with a peptide domain to generate an asparagine-linked N-acetylglucosamine construct; the whole ensemble is then retrieved and deblocked. This allows for fashioning a wide variety of
15 carbohydrate domains, and also benefits from advantages associated with solid-phase synthesis in the carbohydrate-peptide coupling step.

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Brief Description of the Figures

Figure 1 shows glycal assembly leading to neoglycoproteins.

5 Figure 2 shows the synthesis of 4a. Reagents: a) TBDPSCl, imidazole/DMF 84%; b) carbonyldiimidazole, cat. imidazole, THF (65%) c) 5a, di-tert-butylpyridine, AgClO_4 , SnCl_2 , ether (51%); PhSO_2NH_2 , $\text{I}(\text{sym-coll})_2\text{ClO}_4$ (94%).

10 Figure 3 shows the synthesis of 8a. Reagents: a) 9a, AgBF_4 , 4A mol. sieves, THF (75%); b) i. TBAF, THF; ii. Na/NH_3 ; iii Ac_2O , pyr. c) i. 3,3-dimethyloxirane; allyl alcohol, ZnCl_2 (72%); ii. NaOMe, MeOH (quant.).

15 Figure 4 shows a strategy for the solid-phase of oligosaccharides using the glycal assembly method.

20 Figure 5 shows the application of the solid-support method to the assembly of 1,2-branching patterns of complex carbohydrates.

Figure 6 shows the synthesis of a tetrasaccharide having H-type 2 blood group specificity. Reagents: (a) 1. 3,3-dimethyldioxirane, CH_2Cl_2 ; 2. 8, ZnCl_2 , THF; (b) 10, $\text{Sn}(\text{OTf})_2$, di-tert-butylpyridine, THF; (c) TBAF, AcOH, THF; (d) TIPSCl, imidazole, DMF; (e) $\text{I}(\text{coll})_2\text{ClO}_4$, PhSO_2NH_2 , CH_2Cl_2 ; (f) 15, AgBF_4 , 4A M.S., THF; (g) 1. TBAF, AcOH, THF; 2. Na/NH_3 ; 3. Ac_2O , pyridine.

30 Figure 7a and 7b show the synthesis of a Le^b hexasaccharide in bioconjugatable form. Reagents: (a) 1. 3,3-dimethyldioxirane, CH_2Cl_2 ; 2. 19, ZnCl_2 , THF; (b) 10, $\text{Sn}(\text{OTf})_2$, di-tert-butylpyridine, THF; (c) TBAF, AcOH, THF; (d) TIPSCl, imidazole, DMF; (e) $\text{I}(\text{coll})_2\text{ClO}_4$, PhSO_2NH_2 , CH_2Cl_2 ; (f) 24, AgBF_4 , 4A M.S., THF; (g) 1. TBAF, AcOH, THF; 2. Na/NH_3 ; 3. Ac_2O , pyridine; (h) 1. 3,3-dimethyldioxirane, CH_2Cl_2 ; 2. allyl alcohol, ZnCl_2 ; 3. NaOMe, MeOH.

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Figur 8 shows the structure of th MBrl antigen and a reaction pathway to a trisaccharide intermediate.

Reagents: a. n-Bu₂SnO, PMBCl, TBABr, PhH, 70%; b. NaH, BnBr, DMF, 95%; c. (i) 3,3-dimethyldioxirane, CH₂Cl₂; (ii) TBAF, THF; (iii) NaH, BnBr, DMF, 40% (three steps); d. NaH, BnBr, DMF, 80%; e. (i) TBAF, THF; (ii) NaOMe, MeOH, 93% (two steps); f. (n-Bu₃Sn)₂O, BnBr, TBABr, PhH, 90%; g. SnCl₂, AgClO₄, 2,6-di-butylpyridine, 4 Å mol. sieves, Et₂O, 40% & (4.5:1 &:B); h. DDQ, CH₂Cl₂, H₂O, 84%.

10

Figure 9 shows a reaction pathway to a trisaccharide intermediate.

Reagents: a. (i) 3,3-dimethyldioxirane, CH₂Cl₂; (ii) 10a, ZnCl₂, THF, 87%; b. SnCl₂, AgClO₄, Et₂O, 47%; c. I(coll)₂ClO₄, PhSO₂NH₂, 4 Å mol. sieves, 47%.

15

Figure 10(a) shows a reaction pathway to the hexasaccharide MBrl antigen.

Reagents: a. EtSH, LiHMDS, DMF, 75%. B. 8b (0.5 equiv), MeOTf, 4 Å Mol. sieves, 70-85% B, (10:1 B &); c. (i) 3,3-dimethyldioxirane, CH₂Cl₂ (ii) 17b (5 equiv), Zn(OTf)₂, 20%; d. Ac₂O, Et₃N, DMAP, CH₂Cl₂ 95%; e. Lindlar's cat., H₂ palmitic anhydride, EtOAc, 90%; f. (i) TBAF, THF; (ii) NaOMe, MeOH, 94%; g. (i) Na, NH₃, THF; (ii) Ac₂O, Et₃N, DMAP, CH₂Cl₂, 80% h. NaOMe, MeOH, quant.

25

Figure 10(b) shows a reaction pathway to the allyl glycoside.

Reagents: a. TBAF, THF, 94%; b. (i) Na, NH₃, THF; (ii) Ac₂O, Et₃N, DMAP, THF, DMF, 85%; c. (i) 3,3-dimethyldioxirane, CH₂Cl₂, (ii) allyl alcohol, 65% (+ 29% of &-manno isomer); d. NaOMe, MeOH, quant.

30

Figure 11 shows a reaction pathway to intermediates for preparing the hexasaccharide antigen MBrl.

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Figure 12 shows a reaction pathway to the hexasaccharide antigen MBrl by a 4+2 synthetic approach.

5 Figure 13 shows intermediates to prepare trisaccharide pentapeptide 39C (27C).

Figure 14 (Scheme I) shows N-linked glycopeptide synthesis via polymer bound glycals.

10 Figure 14 (Scheme II) shows the preparation of polymer-bound 2-N-acetyl-1 β -amino glucosylamine via azasulfonamidation.

P = saccharide protecting group; "I⁺" = iodonium bis(collidine) perchlorate ($1(\text{coll})_2\text{ClO}_4$); "N₃⁻" =
 15 tetrabutylammonium azide; (a) acylation with acetic anhydride and 4-N,N-dimethylaminopyridine (DMAP); (b) reduction with 1,3-propanedithiol and N,N-diisopropyl-N-ethylamine.

20 Figure 15 shows the solid-phase synthesis of N-linked glycopeptides.

Reagents: (a) dimethyldioxirane, THF, (29); 7C or 8C, zinc chloride, THF; (b) Ac₂O collidine DMAP, THF; (c) Bu₄NF, acetic acid, THF, 40°C; (d) $1(\text{coll})_2\text{ClO}_4$ (30),
 25 AnthrSO₂NH₂, 9C→11C; (e) Bu₄NN₃, THF, 11C→12C; (f) Ac₂O, DMAP, THF, 12C→13C; (g) PhSH, i-Pr₂NEt, THF; (h) 1,3-propanedithiol, i-Pr₂NEt (20), THF, 13C→14C; (i) 11DQ, CH₂Cl₂, 14C + 16C→18C and 14C + 17C→19C; (j) HF pyridine, anisole (31), THF 18C→20C and 19C→21C; (k) Pd(PPh₃)₄,
 30 dimethylbarbituric acid, THF (32); (l) Zn, acetic acid, methanol (32); (m) H₂, Pd(OAc)₂, methanol (33); (n) KCN, methanol: 22C (61% from 20C); 23C (48% from 21C) (34).

35 Figure 16 shows the extension of the peptide portion of the glycopeptide on the polymeric solid support.

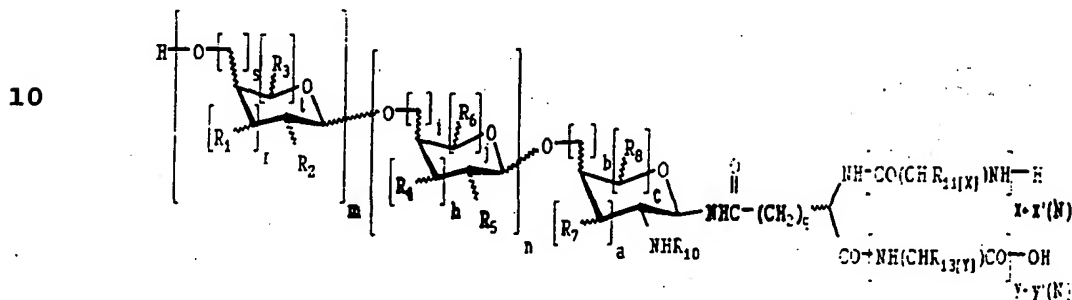
Reagents: (a) 28C, 11DQ, CH₂Cl₂, 14C→24C; (b) Pd(PPh₃)₄, dimethylbarbituric acid, THF 24C→25C; (c) 29C, 11DQ,

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CH_2Cl_2 , 25C→26C; (d) HF, pyridine, anisole, CH_2Cl_2 , 26C→27C.

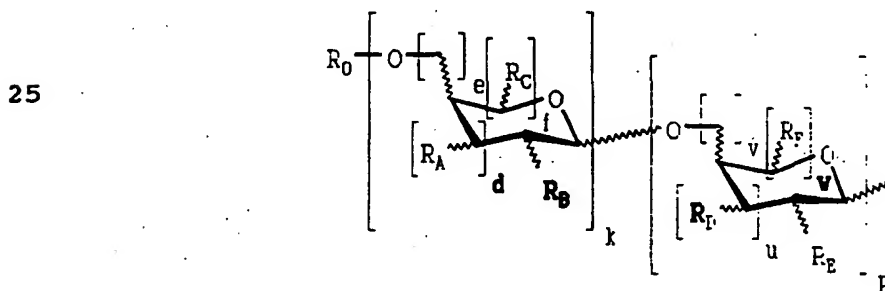
5 Summary of the Invention

The present invention provides a process of synthesizing a glycopeptide having the structure:



wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are each independently H, OH, OR^i , NH_2 , NHCOR^i , F, CH_2OH , CH_2OR^i , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R^i is H, CHO, CO_2R^{ii} , a linear or branched chain alkyl, arylalkyl or aryl group, or an oligosaccharide moiety having the structure:

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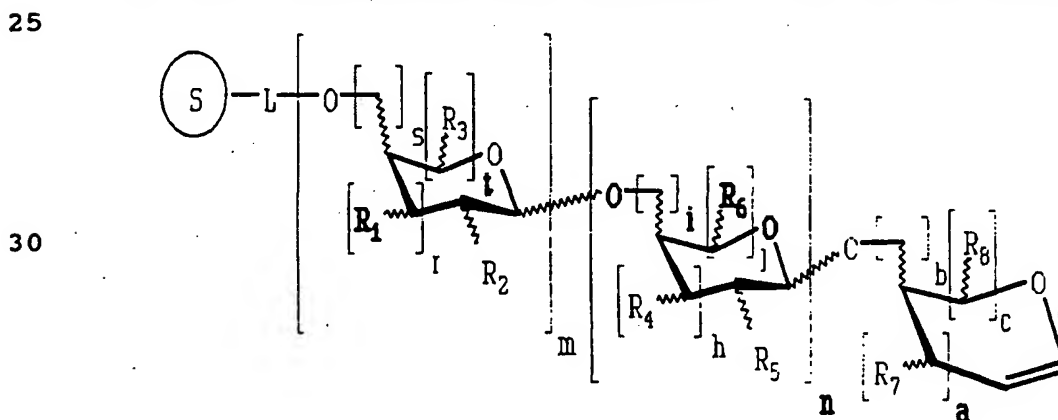
wherein R_0 is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein d, e, f, k, p, u, v and w are each independently 0, 1 or 2; wherein R_A , R_B , R_C , R_D , R_E and R_F are each independently H, OH, OR^{iii} , NH_2 , NHCOR^{iii} , F, CH_2OH , $\text{CH}_2\text{OR}^{iii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl

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or aryl group, wherein R^{iii} is H, CHO, CO_2R^{iv} , a linear or branched chain alkyl, arylalkyl or aryl group, and where in R^{ii} and R^{iv} are independently a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_{10} is a substituted or unsubstituted linear or branched chain acyl, arylacyl or aroyl group; wherein $R_{11[X]}$ represent X amino acid side-chains, where X is an integer from 1 to $x+x'(N)$, and denotes position from the N-terminus, and $x'(N)$ is a summation over N, where N is an integer from 1 to 10, and $R_{13[Y]}$ represent Y amino acid side-chains, where Y is an integer from 1 to $y+y'(N)$, and denotes position from the C-terminus, and $y'(N)$ is a summation over N, where N is an integer from 1 to 10, wherein each $R_{11[X]}$ and $R_{13[Y]}$ are independently the same or different, and are H, OH, a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein a, b, c, h, i, j, r, s and t are each independently an integer between about 0 and about 3; wherein m and n are each independently an integer between about 0 and about 5; wherein q is an integer between about 1 and about 9; and wherein x, $x'(N)$, y and $y'(N)$ are each independently an integer between about 0 and about 25; which comprises:

(a) halosulfonamidating a compound having the structure:



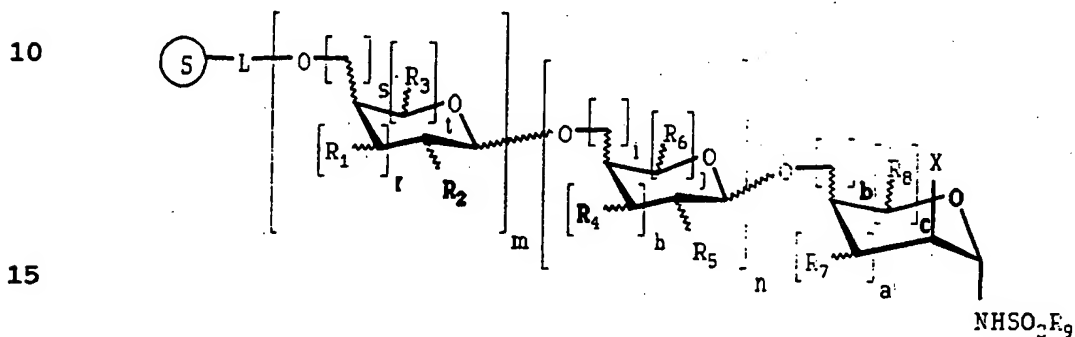
wherein L is a suitable linking moiety selected from the group consisting of SiR_2 , a substituted or unsubstituted

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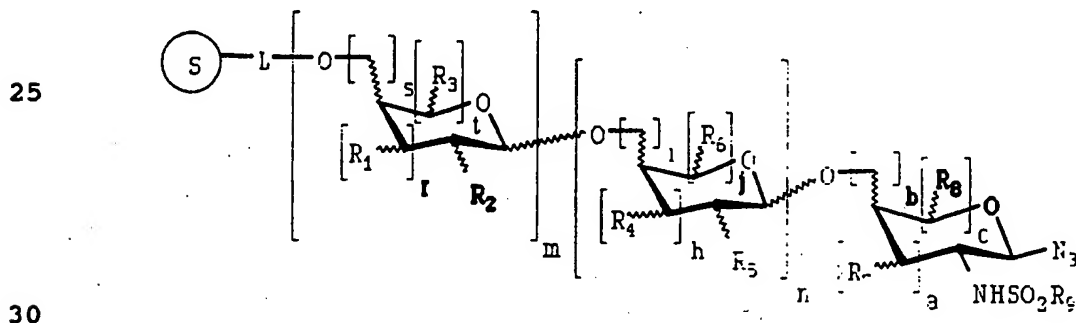
linear or branched chain alkyl, arylalkyl, and aryl groups, where R is a linear or branched chain alkyl, alkoxy, arylalkyl, arylalkoxy or aryl group;

wherein (S) is a polymeric solid-phase; with a compound

- 5 having the formula $R_9SO_2NH_2$, wherein R_9 is a substituted or unsubstituted, or a linear or branched chain alkyl, arylalkyl or aryl group under suitable conditions to form a compound having the structure:

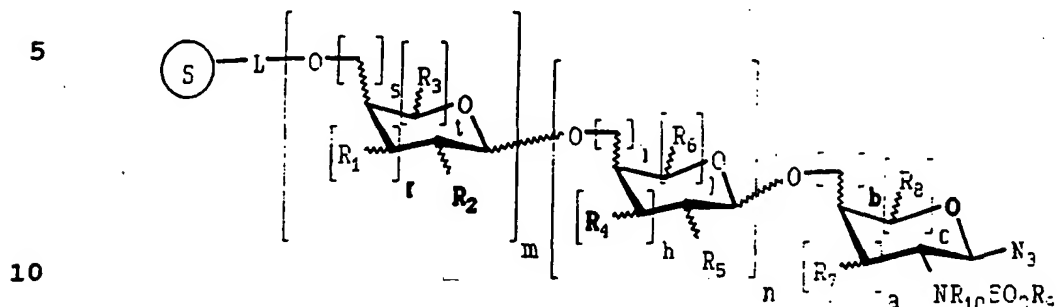


wherein X is selected from the group consisting of F, Cl, Br and I; (b) reacting the compound formed in step (a) with an azide salt under suitable conditions to form a sulfonamide azide having the structure:

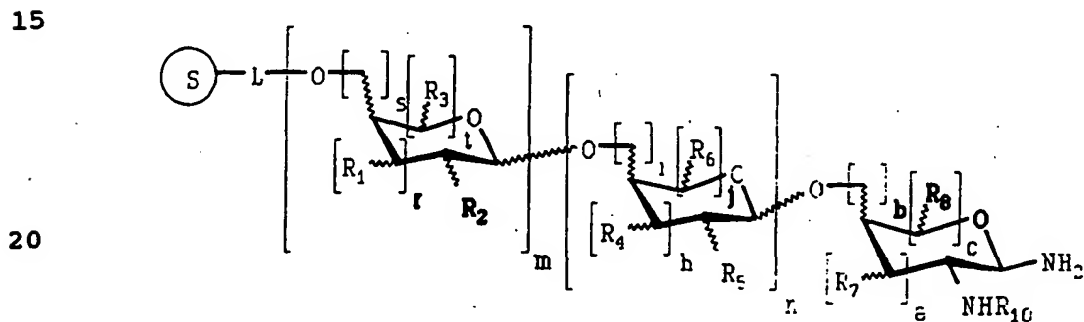


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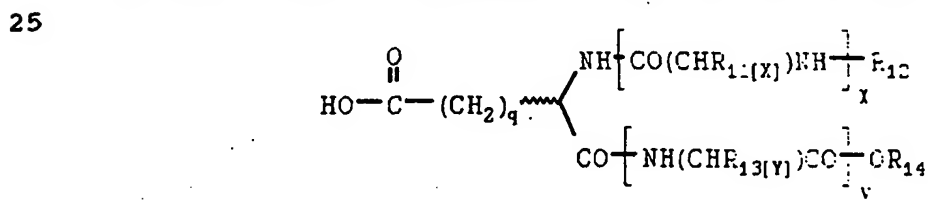
(c) acylating the sulfonamide azide formed in step (b) under suitable conditions to form an N-acylsulfonamide having the structure:



(d) reducing the N-acylsulfonamide formed in step (c) with a reducing agent under suitable conditions to form an amine N-acylamide having the structure:



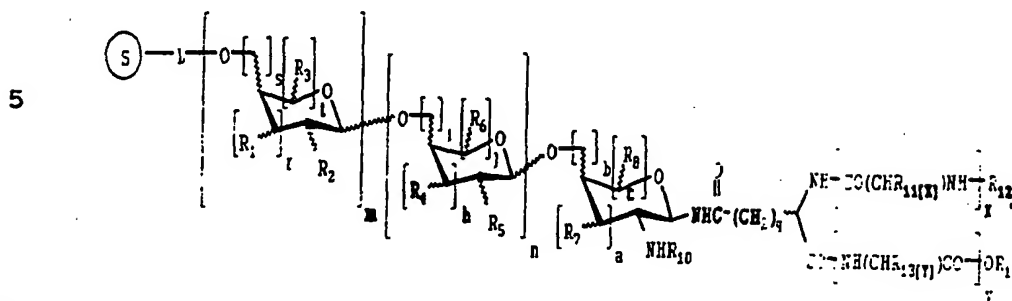
(e) coupling the amine N-acylamide with a suitably protected acidic peptide having the structure:



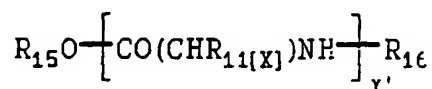
wherein R_{12} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; wherein R_{14} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein X is an integer between about 1

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and x; under suitable conditions to form a protected glycopeptide having the structure:

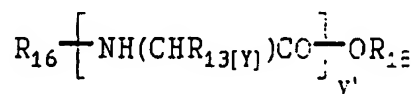


(f) (i) selectively deprotecting the protected glycopeptide formed in step (e) under suitable conditions to form either an N- or C-deprotected glycopeptide; (ii) coupling the N- or C-deprotected glycopeptide
 15 respectively under suitable conditions with a protected amino acid or oligopeptide having the structure:



20 wherein R_{15} is H; and wherein R_{16} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; or having the structure:

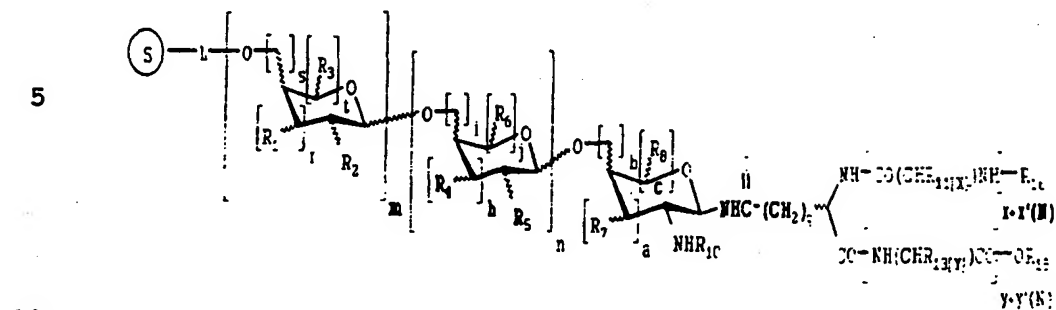
25



30 wherein R_{16} is H; and wherein R_{15} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and (iii) optionally repeating iteratively

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steps (i) and (ii) N times to form a chain-extended glycopeptid having the structure:



and (g) cleaving and deprotecting the chain-extended glycopeptide under suitable conditions to form the glycopeptide.

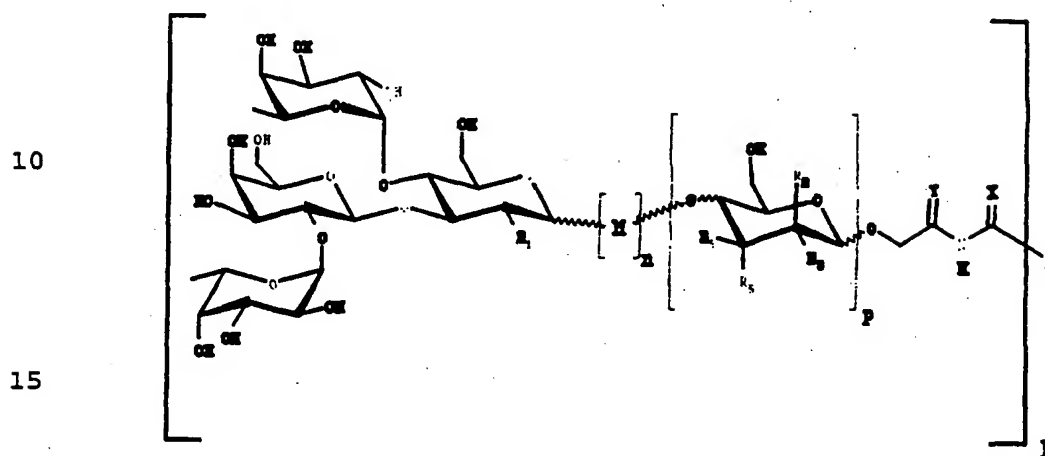
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Detailed Description of the Invention

The present invention provides a compound having the structure:

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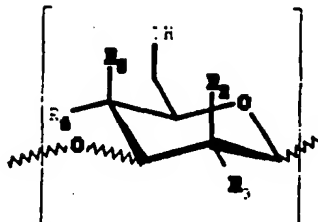


20 wherein A is selected from the group consisting of (i) an amino acid bearing an ω -amino group or an ω -(C=O)- group, (ii) an amino acid residue of a peptide, which residue bears an ω -amino group or an ω -(C=O)- group, and (iii) an amino acid residue of a protein, which residue bears an

25 ω -amino group or an ω -(C=O)- group; wherein R_1 is H, OH, NH_2 , or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain

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alkyl or acyl group, or an aryl group; wherein M has the structure:



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wherein n is an integer from 0 to 18, and where n is greater than 1, each M is independently the same or different; wherein p is either 0 or 1; wherein R_2 , R_3 , R_5 and R_6 are independently the same or different and are H or OH , with the proviso that geminal R_2 and R_3 are not both OH , and geminal R_5 and R_6 are not both OH ; wherein each wavy line between a carbon atom and an oxygen atom denotes an R or S configuration at the carbon atom; wherein X and Y are independently the same or different and are H , or O ; and wherein k is an integer greater than or equal to 1, with the proviso that when A is an amino acid bearing an ω -amino group or an ω -($C=O$)- group, k is equal to 1.

25 In one embodiment, the present invention provides the compound disclosed hereinabove wherein A is lysine or a lysine residue.

30 In another embodiment, the present invention provides the compound disclosed hereinabove wherein A is glutamic acid or a glutamic acid residue.

35 In another embodiment, the present invention provides the compound disclosed hereinabove wherein A is aspartic acid or an aspartic acid residue.

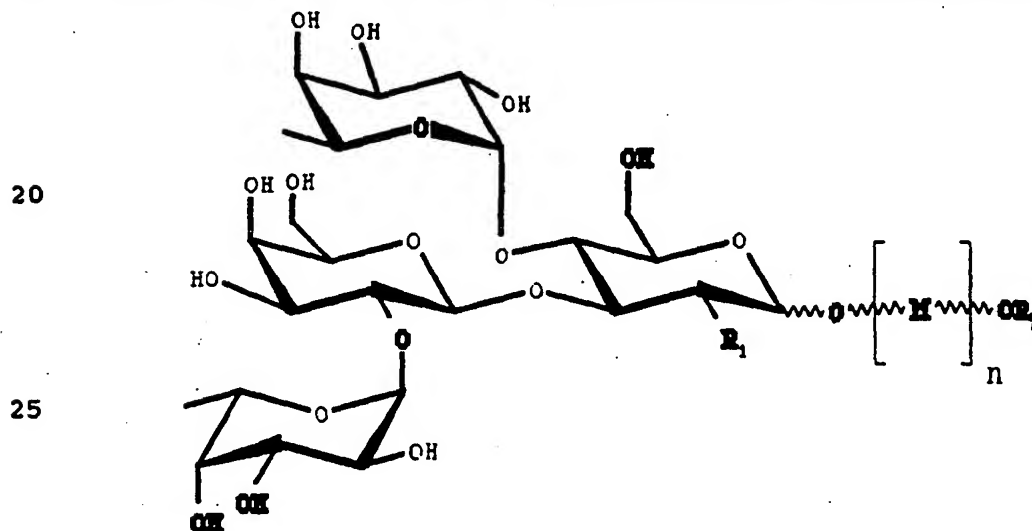
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The invention also provides the compound disclosed hereinabove wherein A is an amino acid residu of a globular protein. In one embodiment, the invention provides the compound wherein the globular protein is selected from the group consisting of bovine serum albumin and human serum albumin.

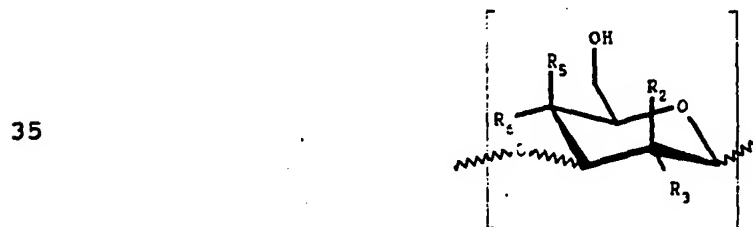
In one embodiment, the invention provides the compound disclosed hereinabove wherein k is 1.

In another embodiment, the invention provides the compound disclosed hereinabove wherein n and p are both equal to 0.

The invention provides a compound having the structure:



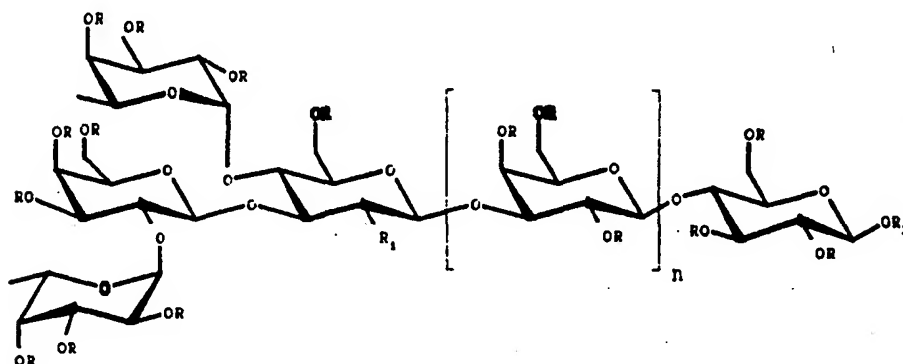
wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl group; wherein M has the structure:



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wherein n is an integer from 0 to 18, and where n is greater than 1, each M is independently the same or different; wherein R_2 , R_3 , R_5 and R_6 are independently the same or different and are H or OH, with the proviso that geminal R_2 and R_3 are not both OH, and geminal R_5 and R_6 are not both OH; wherein each wavy line between a carbon atom and an oxygen atom denotes an R or S configuration at the carbon atom; and wherein R_7 is a substituted or unsubstituted allyl group.

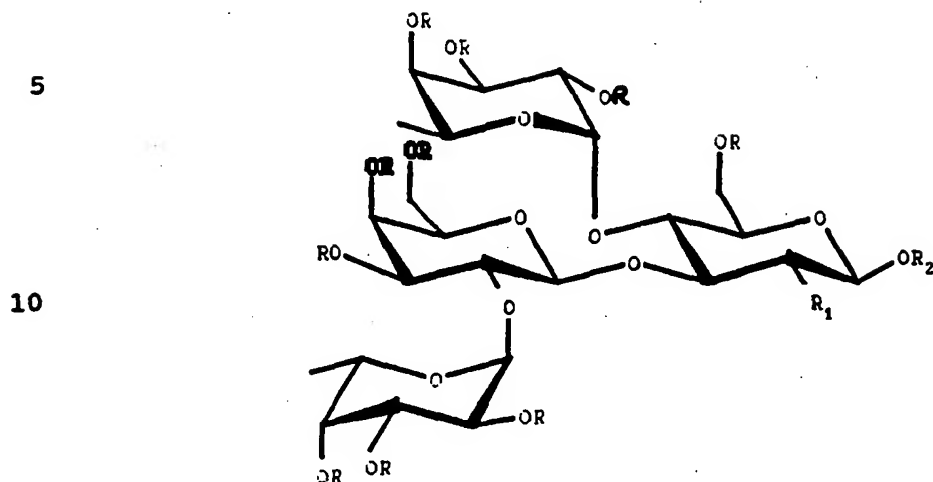
The invention also provides a compound having the structure:



wherein n is an integer from 1 to 18; wherein R is H or a linear or branched chain acyl group; wherein R_1 is H, OH, NH_2 or NHR_4 , where R_4 is SO_2Ph , a linear or branched chain alkyl or acyl group, or an aryl group; and wherein R_2 is a substituted or unsubstituted allyl group. In one embodiment, the invention provides the compound wherein n is 1.

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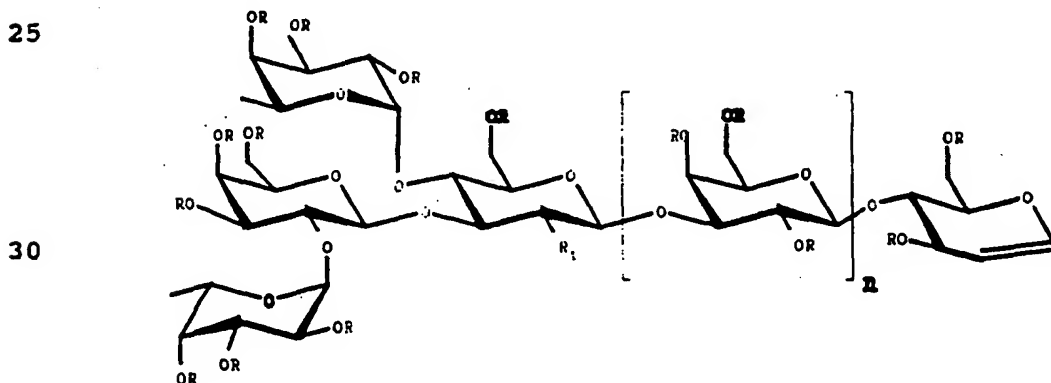
The invention further provides a compound having the structure:



15 wherein R is H or a linear or branched chain acyl group;
wherein R₁ is H, OH, NH₂ or NHR₄, where R₄ is SO₂Ph, a
linear or branched chain alkyl or acyl group, or an aryl
group; and wherein R₂ is a substituted or unsubstituted
allyl group.

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The invention also provides a compound having the structure:

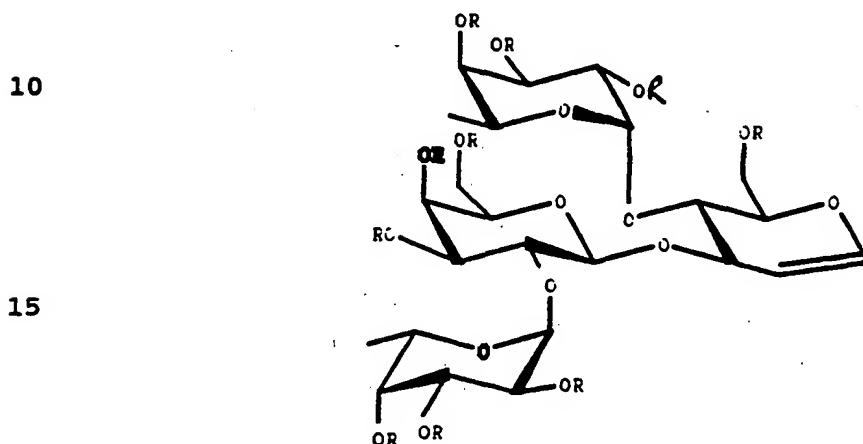


35 wherein R is H or a linear or branched chain acyl group;
wherein R₁ is H, OH, NH₂ or NHR₄, where R₄ is SO₂Ph, a
linear or branched chain alkyl or acyl group, or an aryl

group; wherein R_2 is a substituted or unsubstituted allyl group; and wherein n is an integer from 1 to 18. In one embodiment, the invention provides the compound wherein n is 1.

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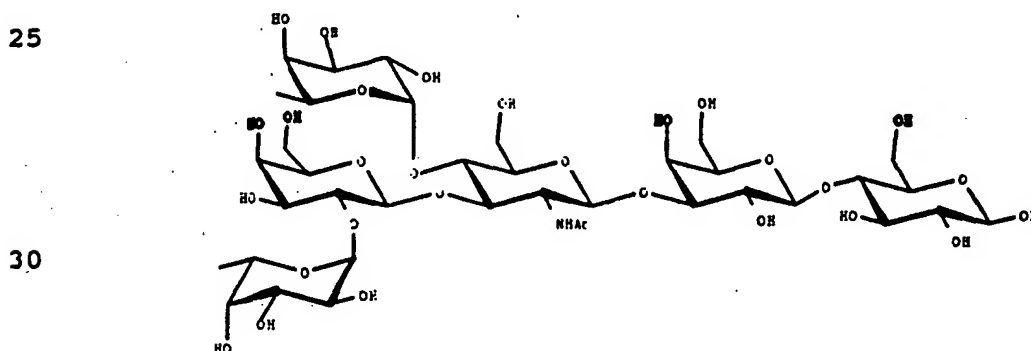
The invention also provides a compound having the structure:



wherein R is H or a linear or branched chain acyl group.

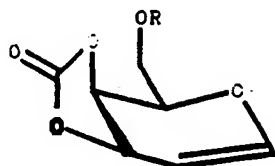
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The invention also provides a process for synthesizing a compound having the structure:

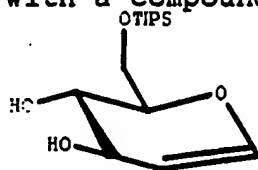


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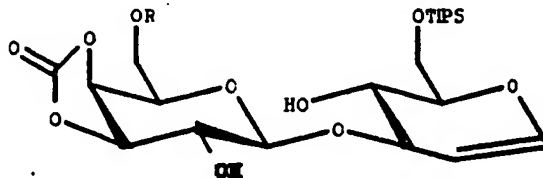
wherein R is a substituted or substituted allyl group, which comprises the steps of (a) synthesizing a compound having the structure:



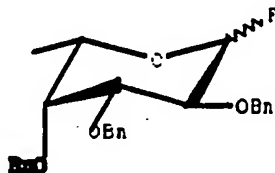
10 wherein R is a trialkylsilyl, aryldialkylsilyl, alkyldiarylsilyl or triaarylsilyl group; (b) reacting the compound of step (a) with a compound having structure:



under suitable conditions to form a compound having the structure:

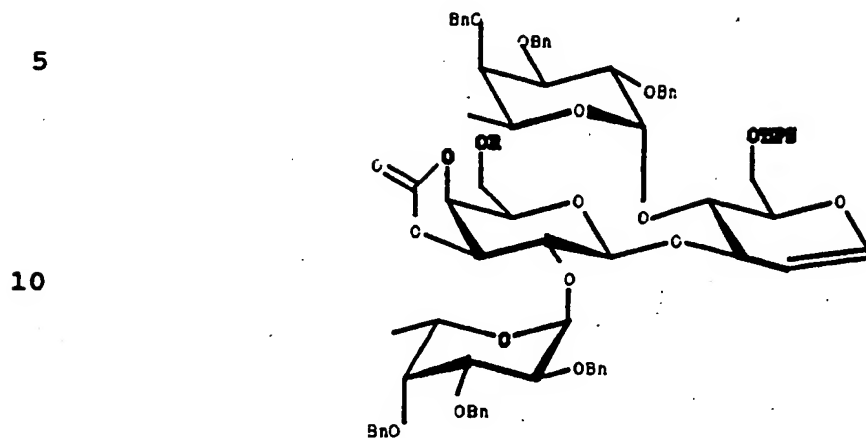


25 wherein R is a trialkylsilyl, aryldialkylsilyl, alkyldiarylsilyl or triaarylsilyl group; (c) reacting the compound formed in step (b) with a compound having the structure:

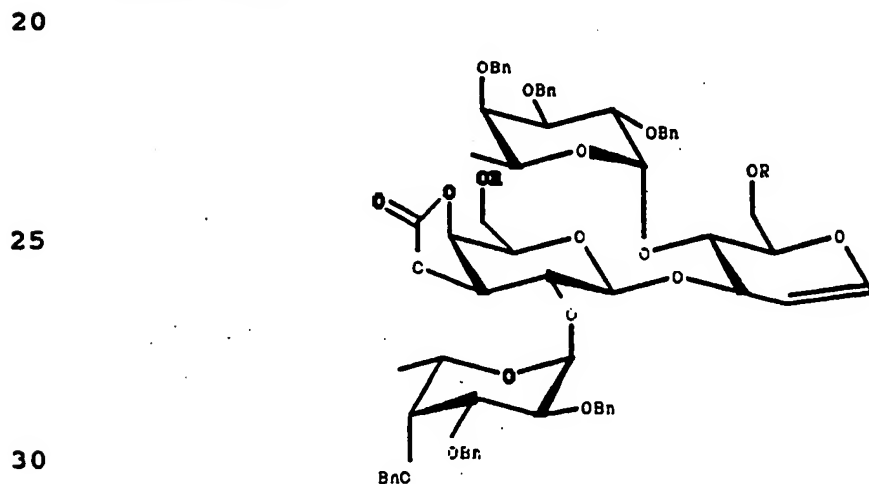


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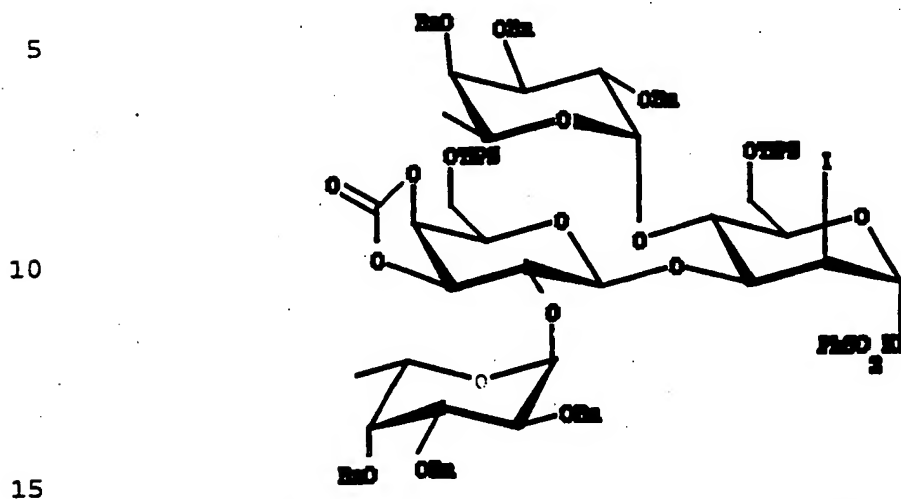
under suitable conditions to form a compound having the structure:



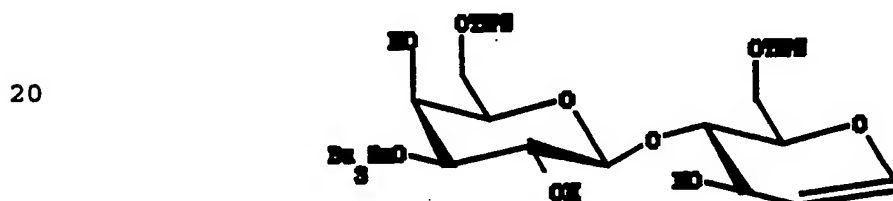
15 wherein R is a trialkylsilyl, aryldialkylsilyl, alkyl diarylsilyl or triarylsilyl group; (d) deprotecting and re-protecting the compound formed in step (c) under suitable conditions to form a compound having the structure:



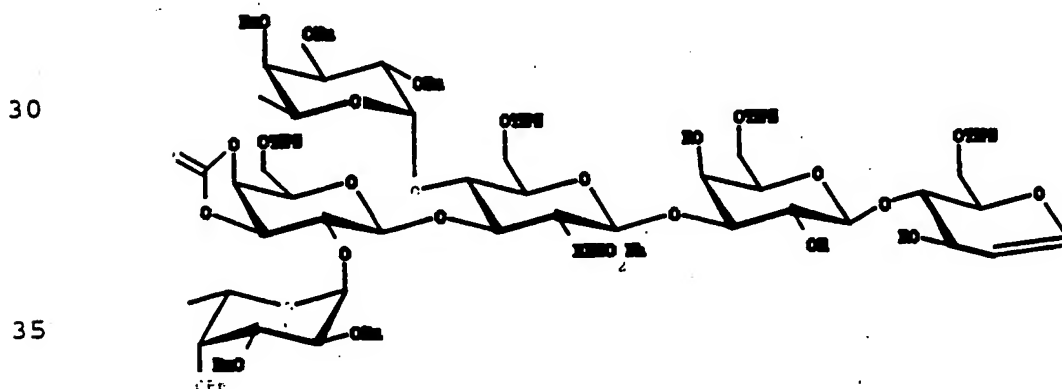
wherein R is TIPS; (e) iodosulfonamidating the compound formed in step (d) under suitable conditions to form a compound having the structure:



(f) reacting the compound formed in step (e) with a compound having the structure:



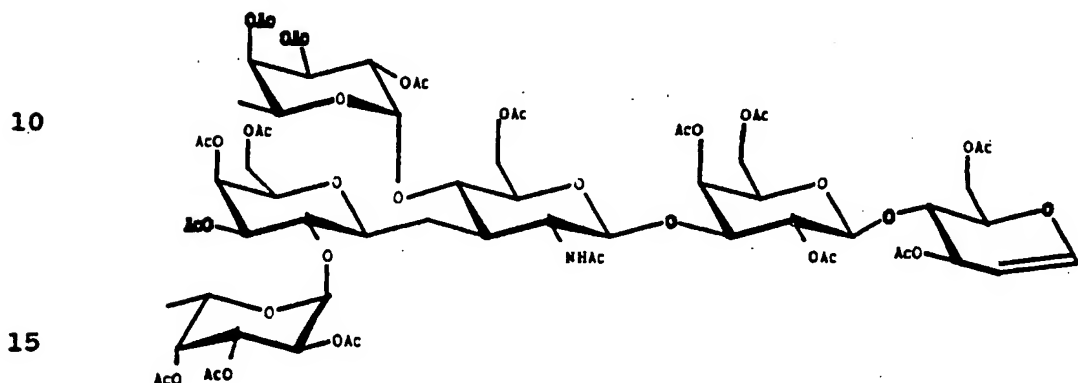
under suitable conditions to form a compound having the structure:



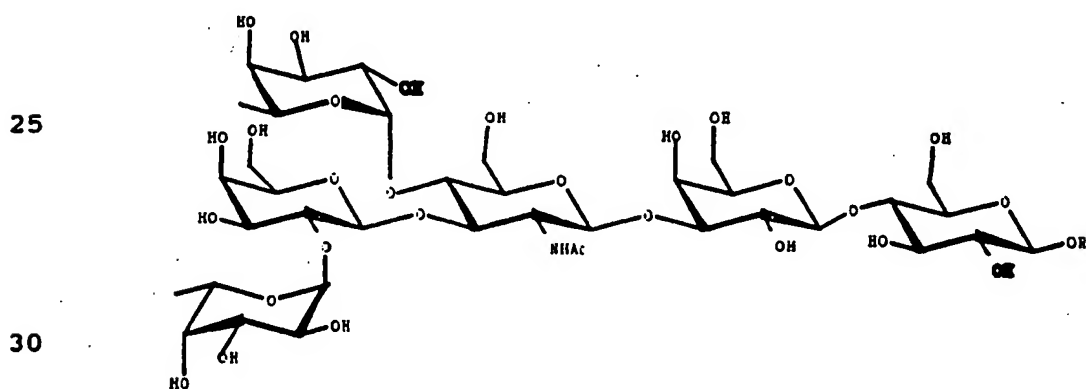
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wherein R is H; (g) deprotecting and peracetylating the compound formed in step (f) under suitable conditions to form a compound having the structure:

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(h) epoxidizing the compound formed in step (g) under suitable conditions to form an epoxide thereof and reacting the epoxide under suitable conditions to form a compound having the structure:



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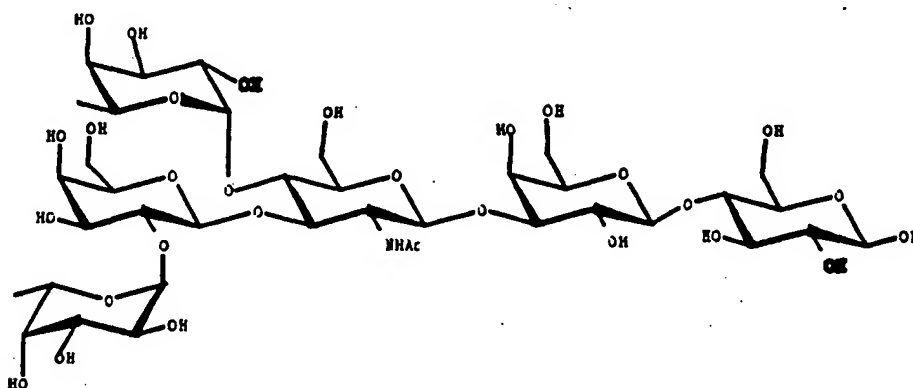
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wherein R is a substituted or unsubstituted allyl group;
and (i) treating the compound formed in step (h) under
suitable conditions to form a compound having the
structure:

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wherein R is a substituted or unsubstituted allyl group.
In the above process the suitable conditions necessary
for the various reactions and treatments may be found in
the Experimental Details section which follows
hereinafter. However, it is within the confines of the
present invention that the specific reagents and solvents
provided as well as the specific conditions necessary for
reaction or treatment may be substituted with other
suitable reactants, solvents and conditions well known to
those skilled in the art.

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The allyl compound may be conjugated to a peptide or
protein via amine or carboxylic acid side chain. In
practicing the invention, a bioconjugate is prepared
according to the protocol of Bernstein and Hall
(Carbohydr. Res. 1980, 78, C1). The allyl group is
ozonolyzed to form either an aldehyde or carboxylic acid,

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which is condensed to a terminal amine to form, respectively, an imine or an amid . The imine is reduced with sodium borohydride to the amine. Alternatively, the aldehyde is reductively aminated using procedures known in the art to form an amine which is reacted with a side-chain terminal carboxylic acid to form an amide conjugate.

The invention provides a pharmaceutical composition which comprises a therapeutically effective amount of the compound disclosed hereinabove and a pharmaceutically acceptable carrier.

Pharmaceutically acceptable carriers are well known to those skilled in the art and include, but are not limited to, 0.01-0.1M and preferably 0.05M phosphate buffer or 0.8% saline. Additionally, such pharmaceutically acceptable carriers may be aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present, such as, for example, antimicrobials, antioxidants, chelating agents, inert gases and the like.

The invention further provides a method for treating a subject afflicted with a disorder caused by Helicobacter pylori which comprises administering to the subject a therapeutically effective amount of the pharmaceutical

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composition disclosed hereinabove so as to treat the subject afflicted with the disorder.

In one embodiment, the invention provides a method of treating a subject afflicted with gastric or duodenal ulcer.

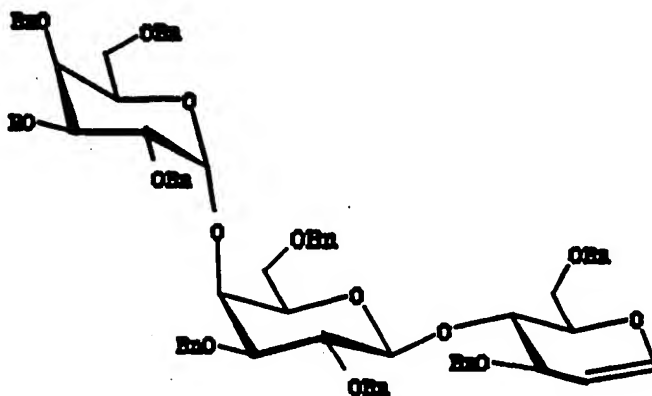
In another embodiment, the invention provides a method of treating a subject afflicted with gastric adenocarcinoma.

In addition, the invention provides a method for inhibiting the adhesion of Helicobacter pylori to gastric epithelium in a subject which comprises administering to the subject an amount of the compound disclosed hereinabove effective to inhibit the adhesion of Helicobacter pylori to gastric epithelium in the subject.

The present invention also provides a process for synthesizing a compound having the structure:

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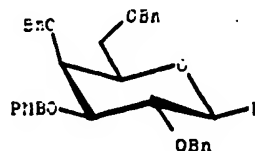
wherein R is H which comprises: (a) (i) reacting a compound having the structure:

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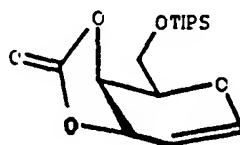


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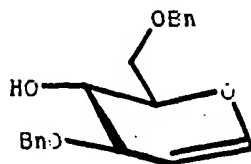
with an epoxidizing agent under suitable conditions to form an epoxide; (ii) cleaving the epoxide formed in step (a)(i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group to form a fluoroalcohol; and (iii) alkylating the fluoroalcohol formed in step (b)(ii) under suitable conditions with a non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F to form a compound having the structure:



(b) (i) synthesizing a compound having the structure:



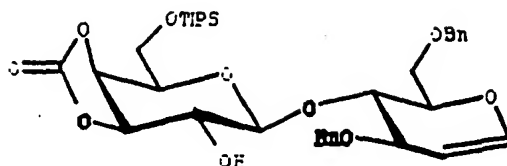
(c) (i) treating the compound formed in step (b) with an epoxidizing agent under suitable conditions to form an epoxide; and (ii) coupling the epoxide formed in step (c)(i) with a compound having the structure:



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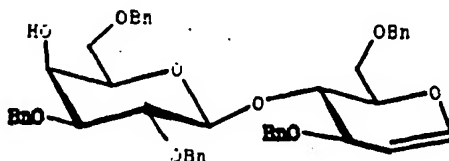
under suitable conditions to form a compound having the structure:

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- (d) (i) alkylating the compound formed in step (c)(ii) under suitable conditions with a non-nucleophilic base and an organic halide having the formula $C_6H_5CH_2X$ wherein X is Br, Cl, I or F; and (ii) de-silylating the compound formed in step (d)(i) under suitable conditions with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group; (iii) treating the compound formed in step (d)(ii) under suitable conditions with a metal alkoxide to form a deprotected disaccharide; and (iv) alkylating the disaccharide formed in step (d)(iii) under suitable conditions to form a selectively deprotected disaccharide having the structure:

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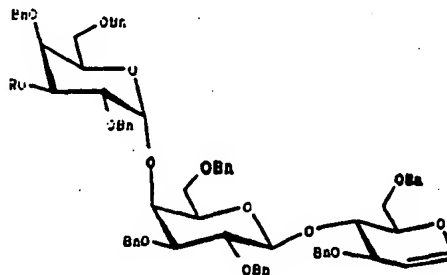


- (e) (i) coupling the selectively deprotected disaccharide formed in step (d)(iv) with the compound formed in step (a)(iii) under suitable conditions to form a protected trisaccharide; and (ii) de-protecting the protected

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trisaccharide formed in step (e)(i) under suitable conditions to form a trisaccharide having the structure:

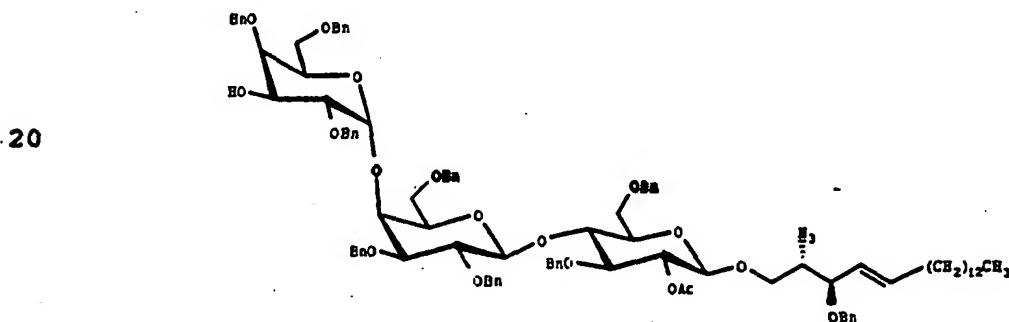


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- 10 wherein R is H. In step (a) reaction (i) may be carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide. A preferred agent is 3,3-dimethyldioxirane. Non-nucleophilic, inert solvents may
- 15 be used, such as dichloromethane. Reaction (a)(ii) may be performed using organic ammonium fluoride salts, including tetrabutylammonium fluoride, in a range of solvents, including ethereal solvents, preferably in tetrahydrofuran. Step (iii) may be performed using a
- 20 non-nucleophilic base such as sodium hydride in a non-nucleophilic solvent such as DMF. In step (b) the compound shown may be prepared as described herein. Step (c)(i) may be carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid,
- 25 trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Coupling step (c)(ii) may be carried out using a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. Step
- 30 (d)(i) is carried out using a non-nucleophilic base such as sodium hydride in a non-nucleophilic solvent such as DMF. In step (d)(ii) de-silylation is effected using an organic ammonium fluoride salt, including tetrabutylammonium fluoride, in a range of solvents,
- 35 including ethereal solvents, preferably in tetrahydrofuran. The carbonate ester is cleaved using a metal alkoxide, such as sodium methoxide, in an alcoholic

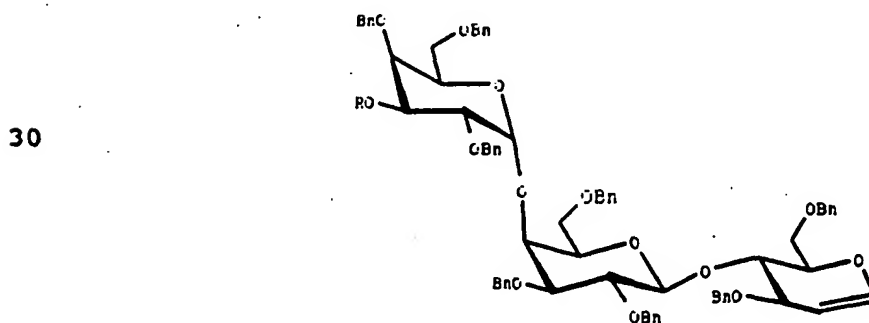
-34-

medium such as methanol. Step (d)(iv) is selectively performed using a metal oxide, such as $(n\text{-Bu}_3\text{Sn})_2\text{O}$, in the presence of an organic ammonium bromide, such as tetra-*n*-butylammonium bromide, in an inert solvent such as benzene. Step (e) is a coupling performed in the presence of a metal halide salt, such as SnCl_2 , in the presence of silver perchlorate and 2,6-di-*t*-butylpyridine, in a solvent, such as ether, containing molecular sieves. Oxidative removal of PMB is performed with an oxidizing agent such as DDQ in an inert solvent system, which may preferably be heterogeneous, for example, using water/dichloromethane.

The present invention also provides a process for synthesizing a trisaccharide ceramide having the structure:



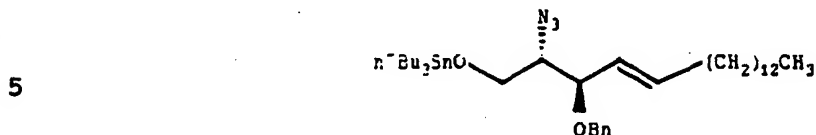
which comprises: (a) synthesizing a trisaccharide having the structure:



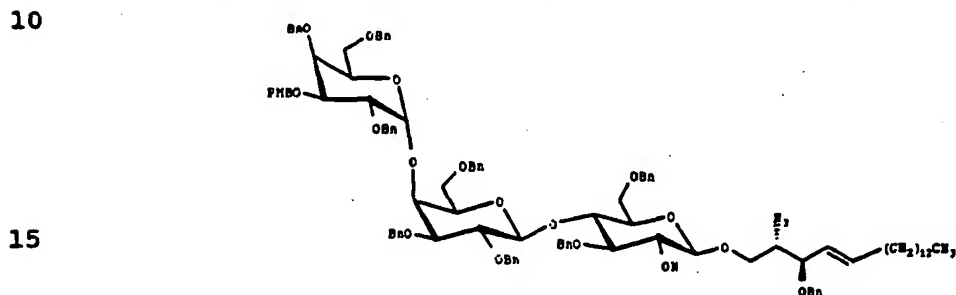
wherein R is PMB; (b) (i) reacting the trisaccharide formed in step (a) with an epoxidizing agent under suitable conditions to form a trisaccharide epoxide; and

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(ii) reacting the trisaccharide epoxide formed in step (b) (i) with a compound having the structure:



under suitable conditions to form a protected trisaccharide ceramide having the structure:



(c) (i) acylating the ceramide formed in step (b)(ii) under suitable conditions; and (ii) selectively de-protecting the compound formed in step (c)(i) under suitable conditions to form the trisaccharide ceramide.

20

In step (a) the trisaccharide may be synthesized as described herein. Step (b)(i) is performed using using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Coupling step (b)(ii) may be carried out using a tributyltin ether of the ceramide precursor and a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. In step (c)(i) acylation is performed using a linear or branched chain alkyl anhydride preferably acetic anhydride or halide in the presence of triethylamine and DMAP in an inert organic solvent such as dichloromethane. The PMB protecting group is removed oxidatively, preferably as described above.

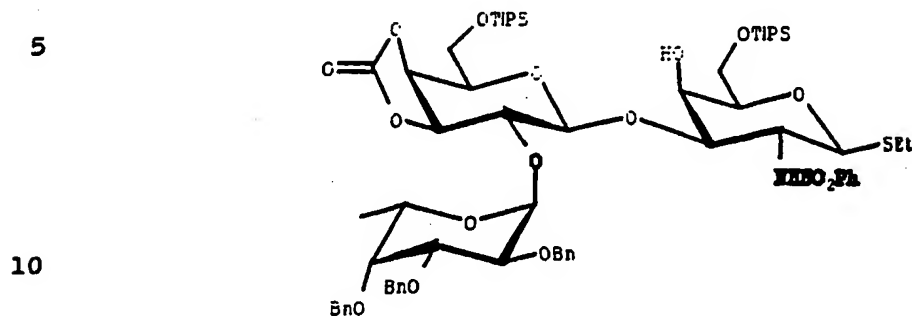
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The present invention further provides a process for synthesizing a mercaptotrisaccharide having the structure:



15 which comprises: (a) (i) synthesizing a compound having the structure:

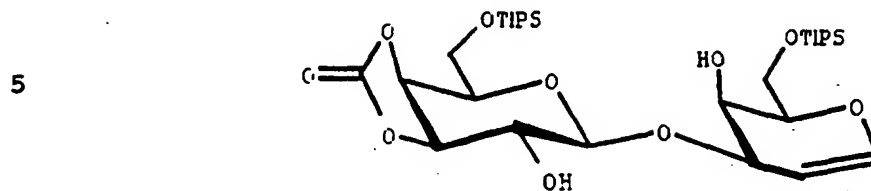


(ii) coupling the compound of step (a)(i) with a compound having structure:

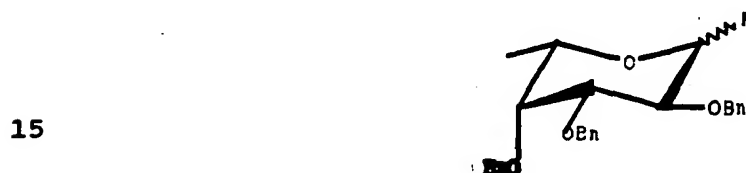


-37-

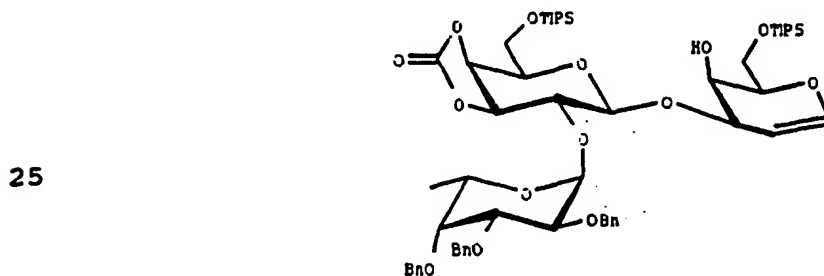
under suitable conditions to form a disaccharide having the structure:



10 (b) coupling the disaccharide formed in step (a)(ii) with a compound having the structure:



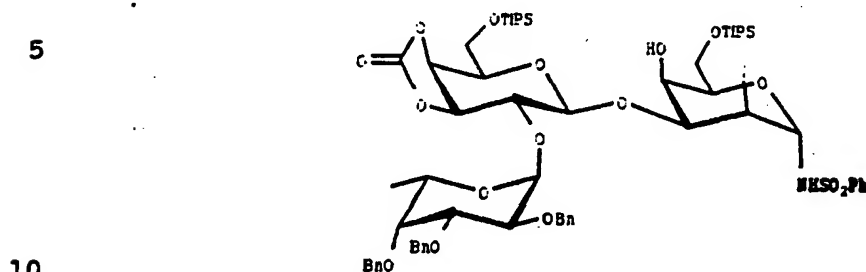
20 under suitable conditions to form a trisaccharide having the structure:



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(c) iodosulfonamidating the trisaccharide formed in step (b) under suitable conditions to form an iodosulfonamide having the structure:



and (d) reacting the iodosulfonamide formed in step (c) under suitable conditions with a thiolate to form the mercaptotrisaccharide.

20 Step (a)(ii) is performed by reacting the compound of step (a)(i), which may be obtained as described herein or otherwise, with a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane, followed by coupling with the diol monosaccharide of step (a)(ii) which may be carried out using a metal catalyst, such as zinc chloride, in an inert solvent, such as THF. Coupling with the fluorosugar is carried out in step (b) in the presence of a metal halide salt, such as SnCl_2 , in the presence of silver perchlorate and 2,6-di-t-butylpyridine, in a solvent, such as ether, containing molecular sieves. Step (c) is performed using $\text{I}(\text{coll})_2$, perchlorate and PhSO_2NH_2 in the presence of molecular sieves. Step (d) is carried out using alkyl thiol and a base such as LiHMDS in an inert solvent as DMF.

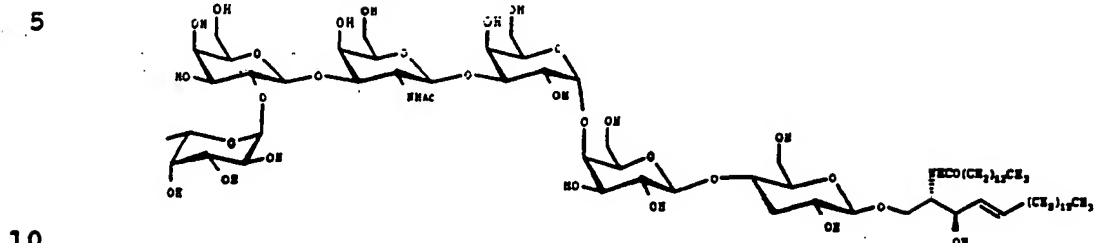
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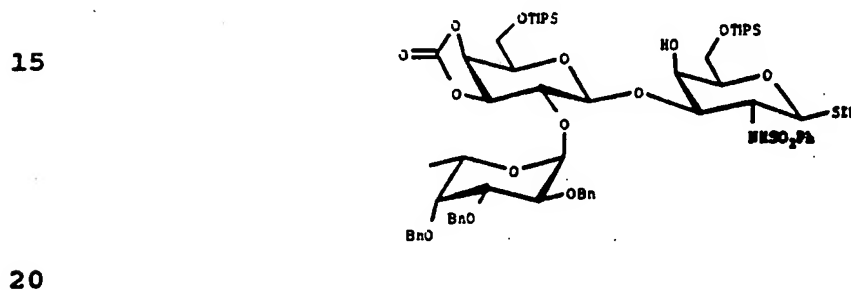
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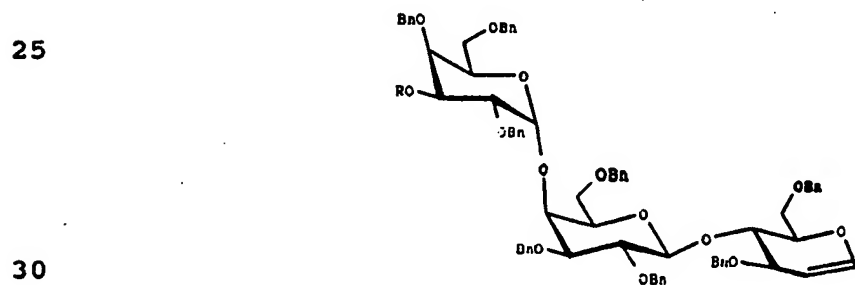
The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:



which comprises: (a) coupling a compound having the structure:

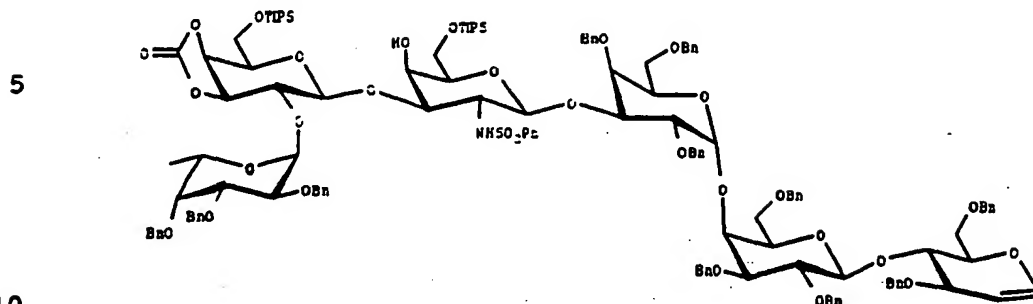


with a compound having the structure:



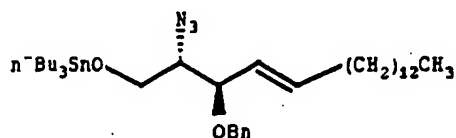
-40-

under suitable conditions to form a compound having the structure:



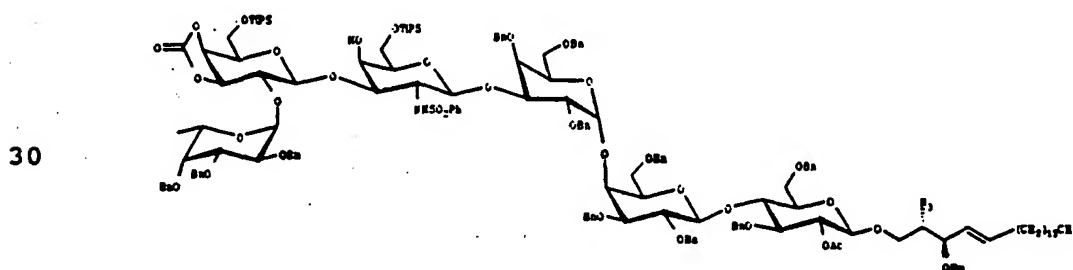
(b) (i) reacting the compound formed in step (a) with an epoxidizing agent under suitable conditions to form a hexasaccharide epoxide; and (ii) reacting the hexasaccharide epoxide with a stannyl ether having the structure:

15



under suitable conditions to form a hexasaccharide alcohol; (c) acylating the hexasaccharide alcohol formed in step (b)(ii) under suitable conditions to form a hexasaccharide acetate having the structure:

25



(d) reductively acylating the hexasaccharide acetate formed in step (c) under suitable conditions in the

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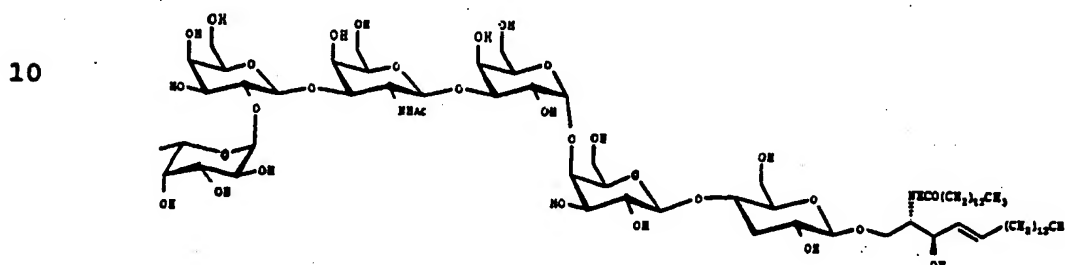
presence of palmitic anhydride to form a hexasaccharide ceramide; (e) desilylating and partially deprotecting the hexasacchararide ceramide under suitable conditions to form a partially deprotected hexasaccharide ceramide; (f) (i) reducing the partially deprotected hexasaccharide ceramide under suitable conditions to form a deprotected hexasaccharide ceramide acetate; and (ii) acylating the deprotected hexasaccharide ceramide acetate under suitable conditions to form a hexasaccharide ceramide peracetate; and (g) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide.

Step (a) is performed using triflate esters, such as methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Step (b)(ii) is performed using a stannyl ether of the ceramide precursor, preferably the tri-n-butyl stannylether, in the presence of a metal salt, such as Zn triflate, in an inert solvent, such as THF. Step (c) is carried out using acetic anhydride in the presence of a base such as triethylamine and DMAP. Step (d) is carried out using a noble metal catalyst such as Lindlar's catalyst and hydrogen gas in the presence of palmitic anhydride in an inert solvent such as ethyl acetate. Desilylation step (e) is effected using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. The carbonate ester is cleaved using a metal alkoxide such as NaOMe in an alcohol such as methanol. In step (f)(i) reduction is performed using a metal such as lithium or sodium in liquid ammonia and an inert solvent such as THF. Step (f)(ii) is carried out using acetic anhydride in the presence of a base such as Et₃N and DMAP

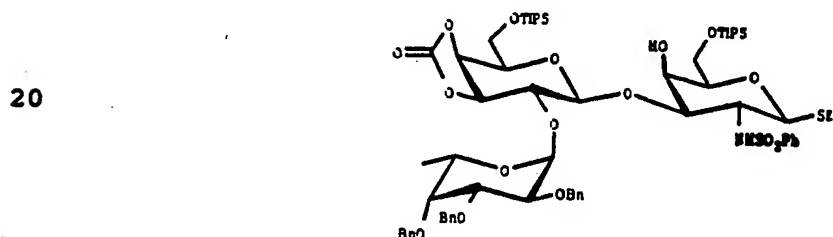
-42-

in an inert solvent such as dichloromethane. The peracetate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

- 5 The present invention also provides a process of synthesizing a hexasaccharide ceramide having the structure:

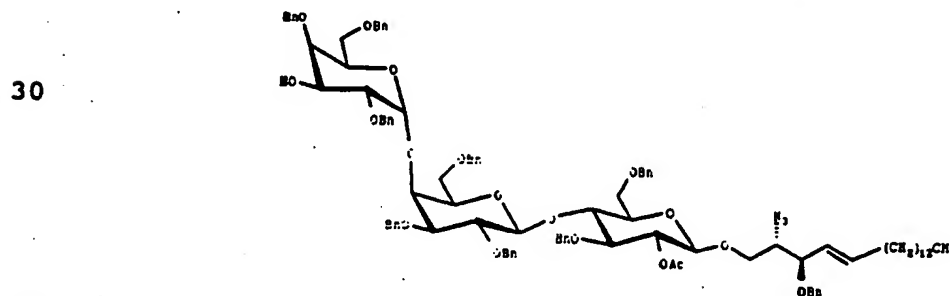


- 15 which comprises: (a) coupling a compound having the structure:



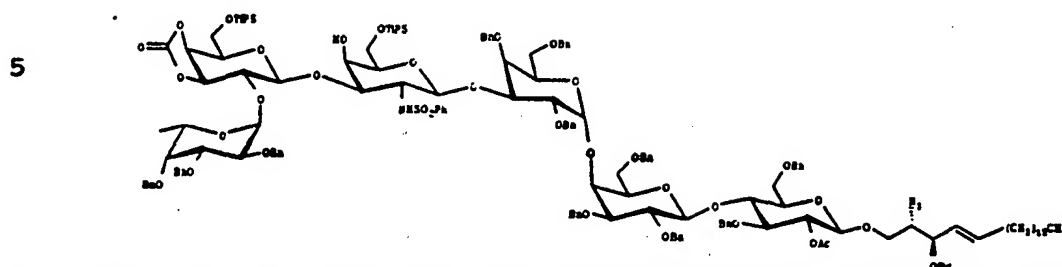
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with a compound having the structure:



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under suitable conditions to form a hexasaccharide having the structure:



- 10 and (b) (i) reducing the hexasaccharide formed in step (a) under suitable conditions in the presence of palmitic anhydride to form a palmitoyl amide; (ii) desilylating the palmitoyl amide with R_4NF wherein each R is independently the same or different and is a linear or
- 15 branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide; (iii) de-protecting the hexasaccharide formed in step (b)(ii) under suitable conditions to form a deprotected hexasaccharide; (iv) acylating the
- 20 hexasaccharide formed in step (b)(iii) under suitable conditions to form a hexasaccharide ceramide peracetate; and (v) saponifying the hexasaccharide ceramide peracetate under suitable conditions to form the hexasaccharide ceramide.

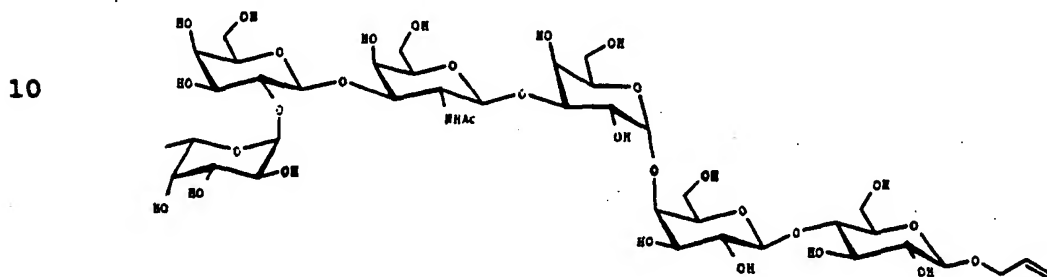
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- Step (a) is performed using triflate esters, such as methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using using a noble metal catalyst such as Lindlar's catalyst and
- 30 hydrogen gas in the presence of palmitic anhydride in an inert solvent such as ethyl acetate. Step (b)(ii) is performed using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. In step (b)(iii) reduction is performed using a metal such as lithium or
- 35 sodium in liquid ammonia and an inert solvent such as THF. Step (b)(iv) is carried out using acetic anhydride in the presence of a base such as Et_3N and DMAP in an

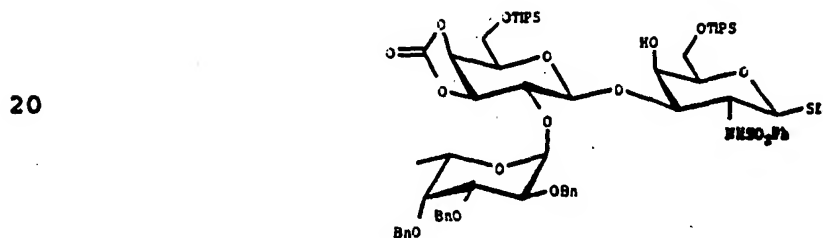
-44-

inert solvent such as dichloromethan . In step (v) the peracetate carbonate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

- 5 The present invention also provides a process of synthesizing an allyl hexasaccharide having the structure:

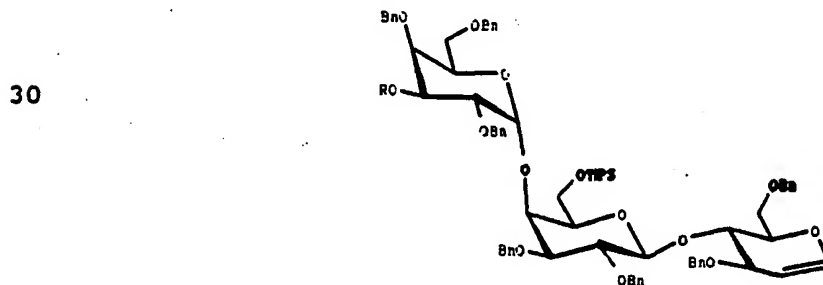


- 15 which comprises: (a) coupling a compound having the structure:



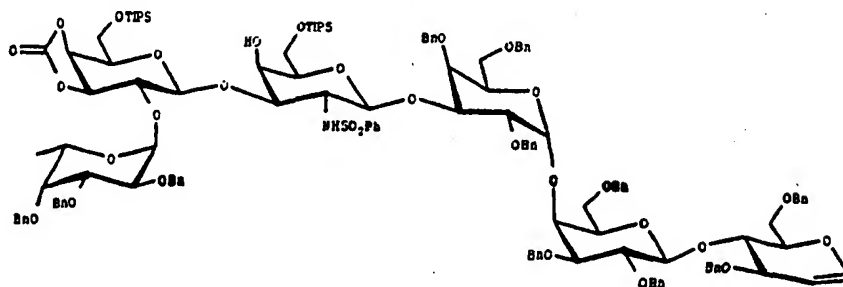
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with a compound having the structure:

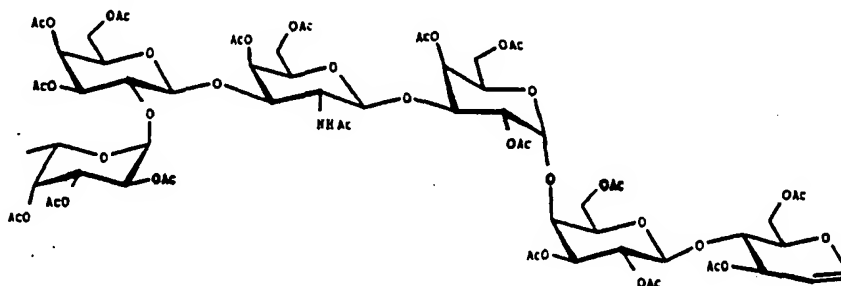


-45-

wherein R is H under suitable conditions to form a hexasaccharide having the structure:



(b) (i) desilylating the compound formed in step (a) with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions to form a partially deprotected hexasaccharide; (ii) de-protecting the hexasaccharide formed in step (b)(i) under suitable conditions to form a deprotected hexasaccharide; and (iii) peracetylating the compound formed in step (b)(ii) under suitable conditions to form a hexasaccharide peracetate having the structure:



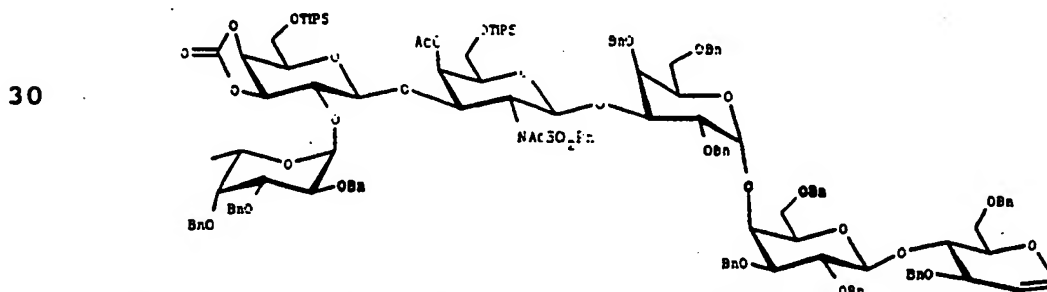
(c) (i) reacting the hexasaccharide peracetate formed in step (b)(iii) with an epoxidizing agent under suitable conditions to form an hexasaccharide epoxide peracetate; (ii) treating the hexasaccharide epoxide peracetate formed in step (c)(i) with allyl alcohol under suitable conditions to form an allyl hexasaccharide peracetate; and (iii) saponifying the allyl hexasaccharide peracetate

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under suitable conditions to form the allyl hexasaccharide.

Step (a) is performed using triflate esters, such as methyl triflate, in the presence of molecular sieves in an inert solvent. Step (b)(i) is carried out using organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. Step (b)(ii) is performed using a metal alkoxide such as sodium methoxide in an alcohol such as methanol, followed by reduction performed using a metal such as lithium or preferably sodium in liquid ammonia and an inert solvent such as THF. Step (b)(iii) is carried out using acetic anhydride in the presence of a base such as Et_3N and DMAP in an inert solvent such as dichloromethane. In step (c)(i) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Step (c)(ii) is carried out using allyl alcohol in an inert solvent. Step (c)(iii) the peracetate carbonate is saponified using a metal alkoxide such as sodium methoxide in an alcohol such as methanol.

The present invention provides a process of synthesizing a hexasaccharide having the structure:

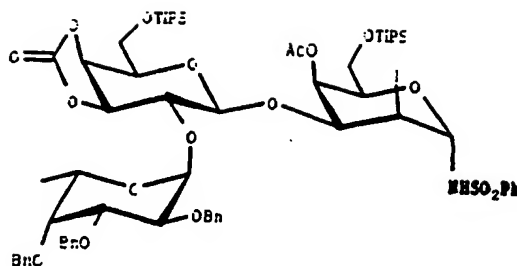


which comprises: (a) coupling a compound having the

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structure:

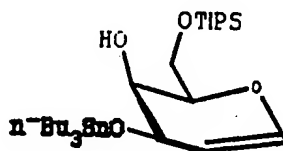
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with a compound having the structure:

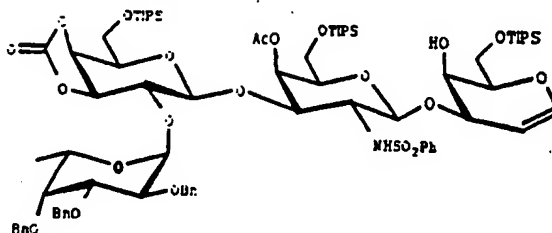
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under suitable conditions to form a compound having the structure:

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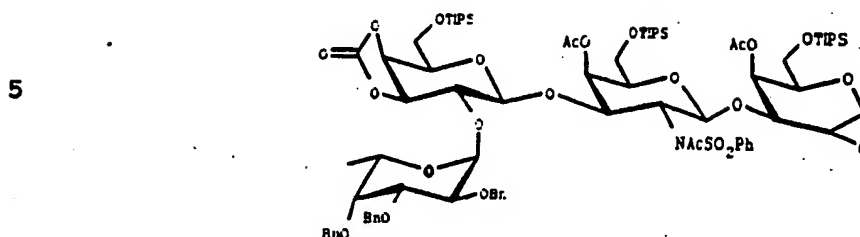
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(b) (i) acylating the compound formed in step (a) under suitable conditions; and (ii) reacting the compound formed in step (b)(i) with an epoxidizing agent under

35

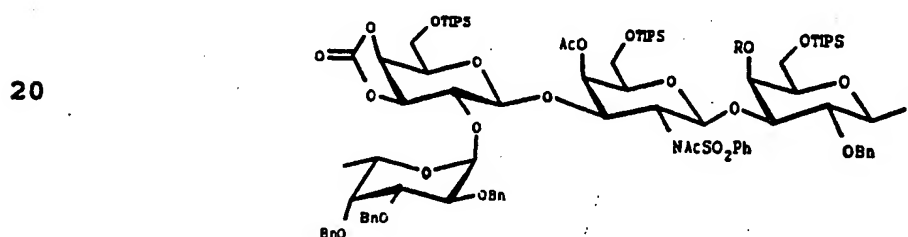
-48-

suitable conditions to form an epoxide having the structure:

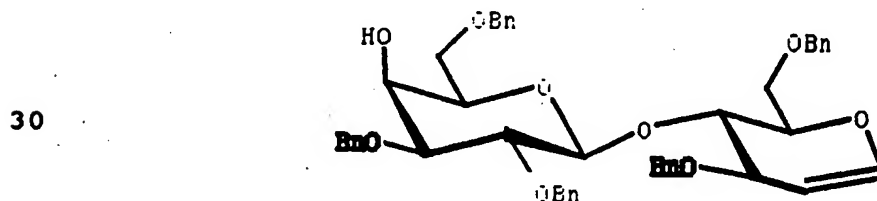


10

(c) (i) treating the epoxide with R_4NF wherein each R is independently the same or different and is a linear or branched chain alkyl, aralkyl or aryl group under suitable conditions; and (ii) alkylating the compound formed in step (c)(i) under suitable conditions to form a compound having the structure:



25 wherein R is H or acyl; (d) coupling the compound formed in step (c)(ii) with a compound having the structure:



35 under suitable conditions to form the hexasaccharide.

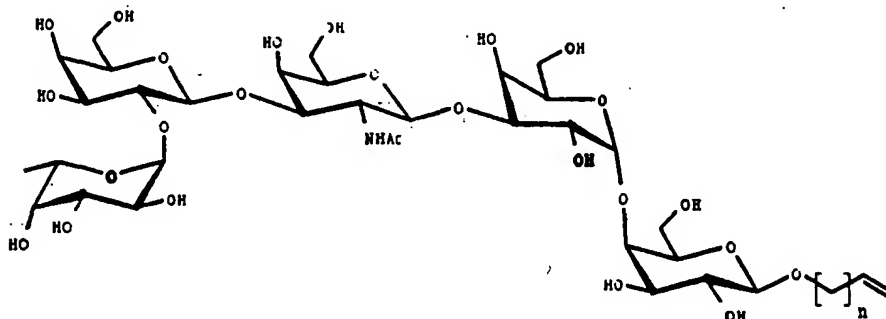
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Step (a) is performed using a metal catalyst such as silver tetrafluoroborate in an inert solvent. Step (b)(i) is carried out using acetic anhydride in the presence of a base such as Et₃N and DMAP in an inert solvent such as dichloromethane. Step (b)(ii) is carried out using a variety of epoxidizing agents including peracetic acid, m-chlorobenzoic acid, trifluoroacetic acid, and hydrogen peroxide, 3,3-dimethyldioxirane being preferred, in non-nucleophilic, inert solvents, such as dichloromethane. Step (c)(i) is effected with organic ammonium fluoride salts, such as tetra-n-butylammonium fluoride in THF. Step (c)(ii) is performed using a non-nucleophilic base such as sodium hydride in an inert solvent. Step (d) is performed using a metal salt catalyst such as tin dichloride in the presence of silver perchlorate in an inert solvent such as di-t-butylpyridine. Further transformations provide deprotected products or conjugates with proteins or other carriers.

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The present invention further provides a compound having the structure:

25



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wherein n is an integer between about 0 and about 9.

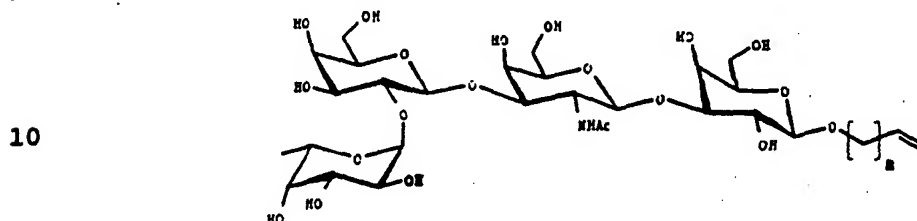
The allyl glycoside shown is prepared using the glycal coupling methods taught herein, and may be bound to protein carriers using general reactions described herein or by standard methods in the art. For example, the allyl glycoside may be prepared by coupling compound 9b

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disclosed herein with a suitably protected 8b, followed by coupling with 12b, then coupling with allyl alcohol and an appropriate deprotection sequence.

- 5 The present invention also provides a compound having the structure:

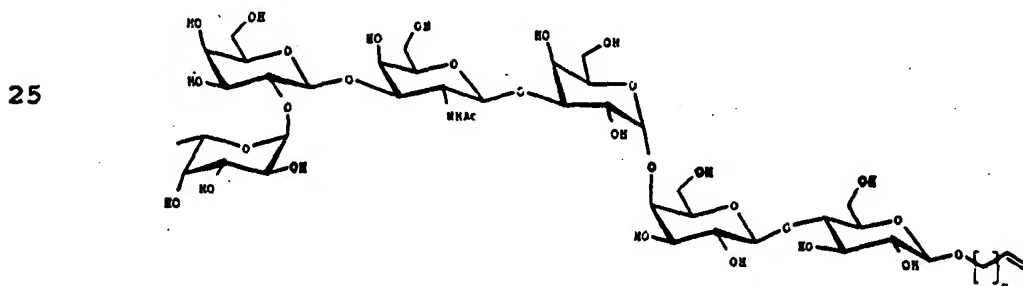


wherein n is an integer between about 0 and about 9.

- 15 The allyl glycoside shown is prepared using the glycal coupling methods, allylation and a deprotection sequence as taught herein (see Fig. 12), and may be bound to protein carriers using general reactions described herein or by standard methods in the art.

20

The present invention also provides a compound having the structure:



- 30 wherein n is an integer between about 0 and about 9.

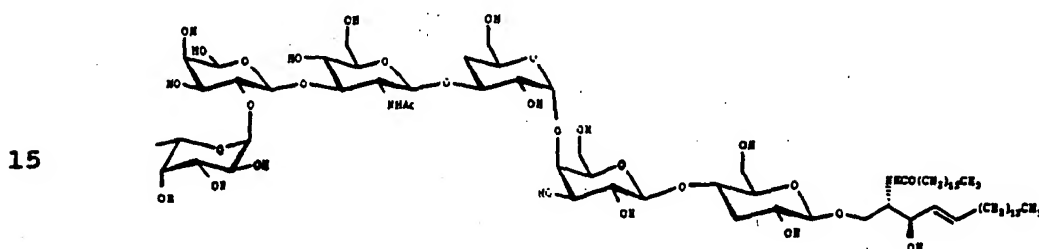
The allyl glycosides shown are prepared using the glycal coupling methods taught herein, and may be bound to protein carriers using general reactions described herein or by standard methods in the art.

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It is within the scope of the present invention to vary the combination of protecting groups for the various sugar hydroxyl groups in accord with ordinary skill in the art.

5 The present invention provides a method of inducing antibodies in a human subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of a
10 compound having the structure:

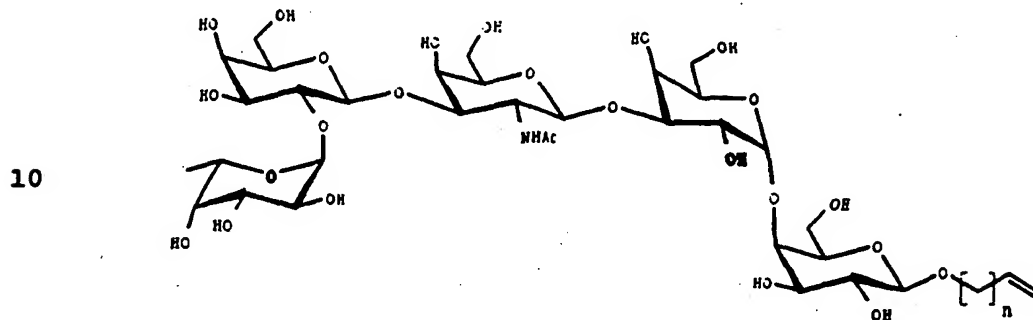


alone or bound to a suitable immunological adjuvant effective to induce the antibodies. In one embodiment,
20 the present invention provides a method wherein the antibodies induced are MBrl antibodies. In another embodiment, the present invention provides a method wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited
25 unresected disease. In another embodiment, the present invention provides a method wherein the adjuvant is a protein carrier, bacteria or liposomes. In yet another embodiment, the present invention provides wherein the adjuvant is bacille Calmette-Guerin (BCG).

30 The present invention provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable
35 immunological carrier, adjuvant or vehicle.

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The present invention also provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which comprises administering to the subject an amount of the compound having the structure:



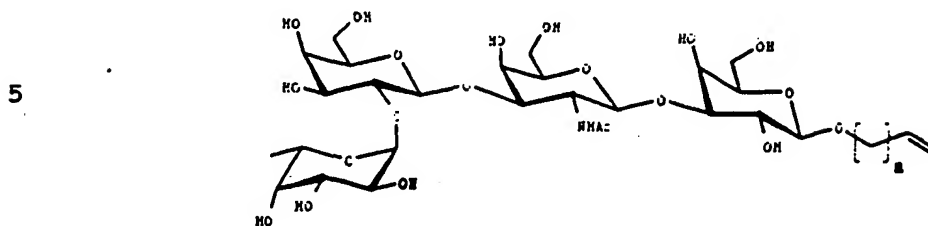
15 wherein n is an integer between about 0 and about 9
either alone or bound to a suitable immunological
adjuvant effective to induce the antibodies. In one
embodiment, the present invention provides a method
wherein the antibodies induced are MBrl antibodies. In
20 another embodiment, the present invention provides a
method wherein the subject is in clinical remission or,
where the subject has been treated by surgery, has
limited unresected disease. In another embodiment, the
present invention provides a method wherein the adjuvant
25 is a protein carrier, bacteria or liposomes. In yet
another embodiment, the present invention provides
wherein the adjuvant is bacille Calmette-Guerin.

30 The present invention provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable immunological carrier, adjuvant or vehicle.

35 The present invention also provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which

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comprises administering to the subject an amount of the compound having the structure:



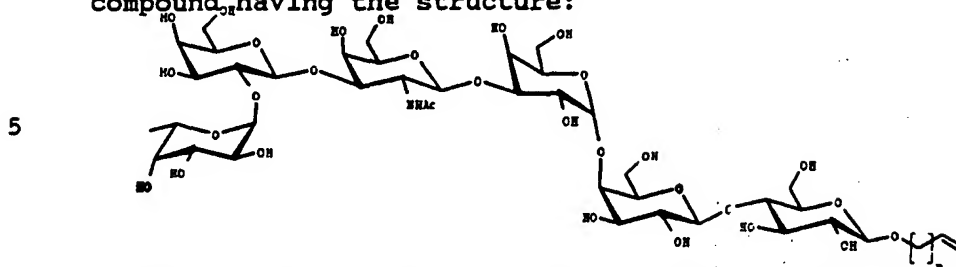
10 wherein n is an integer between about 0 and about 9 either alone or bound to a suitable immunological adjuvant effective to induce the antibodies. In one embodiment, the present invention provides a method wherein the antibodies induced are MBr1 antibodies. In another embodiment, the present invention provides a method wherein the subject is in clinical remission or, where the subject has been treated by surgery, has limited unresected disease. In another embodiment, the present invention provides a method wherein the adjuvant is a protein carrier, bacteria or liposomes. In yet another embodiment, the present invention provides wherein the adjuvant is bacille Calmette-Guerin.

25 The present invention also provides a method of preventing recurrence of breast cancer in a subject which comprises vaccinating the subject with the compound shown hereinabove either alone or bound to a suitable immunological carrier, adjuvant or vehicle.

30 The present invention additionally provides a method of inducing antibodies in a subject, wherein the antibodies are immunoreactive with human breast tumor cells, which

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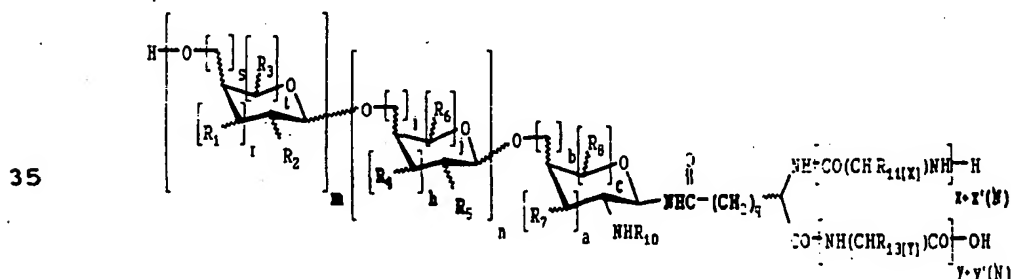
comprises administering to the subject an amount of the compound having the structure:



wherein n is an integer between about 0 and about 9
 10 either alone or bound to a suitable immunological
 adjuvant effective to induce the antibodies. In one
 embodiment, the present invention provides a method
 wherein the antibodies induced are MBrl antibodies. In
 another embodiment, the present invention provides a
 15 method wherein the subject is in clinical remission or,
 where the subject has been treated by surgery, has
 limited unresected disease. In another embodiment, the
 present invention provides a method wherein the adjuvant
 is a protein carrier, bacteria or liposomes. In yet
 20 another embodiment, the present invention provides
 wherein the adjuvant is bacille Calmette-Guerin.

The present invention also provides a method of
 preventing recurrence of breast cancer in a subject which
 25 comprises vaccinating the subject with the compound shown
 hereinabove either alone or bound to a suitable
 immunological carrier, adjuvant or vehicle.

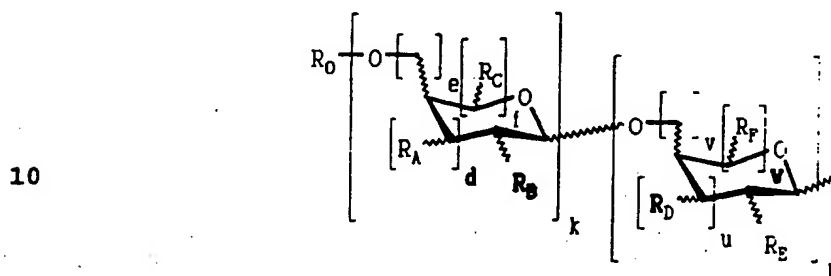
The present invention provides a process of synthesizing
 30 a glycopeptide having the structure:



wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are each
 40 independently H, OH, OR^1 , NH_2 , $NHCOR^1$, F, CH_2OH , CH_2OR^1 ,

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or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R^i is H, CHO, CO_2R^{ii} , a linear or branched chain alkyl, arylalkyl or aryl group, or an oligosaccharide moiety having the structure:



15 wherein R_0 is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein d, e, f, k, p, u, v and w are each independently 0, 1 or 2; wherein R_A , R_B , R_C , R_D , R_E and R_F are each independently H, OH, OR^{iii} , NH_2 , NHCOR^{iii} , F, CH_2OH , $\text{CH}_2\text{OR}^{iii}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, wherein R^{iii} is H, CHO, CO_2R^{iv} , a linear or branched chain alkyl, arylalkyl or aryl group, and wherein R^{ii} and R^{iv} are independently a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_{10} is a substituted or unsubstituted linear or branched chain acyl, arylacyl or aroyl group; wherein $R_{11}[X]$ represent X amino acid side-chains, where X is an integer from 1 to $x+x'(N)$, and denotes position from the N-terminus, and $x'(N)$ is a summation over N, where N is an integer from 1 to 10, and $R_{13}[Y]$ represent Y amino acid side-chains, where Y is an integer from 1 to $y+y'(N)$, and denotes position from the C-terminus, and $y'(N)$ is a summation over N, where N is an integer from 1 to 10, wherein each $R_{11}[X]$ and $R_{13}[Y]$ are independently the same or different, and are H, OH, a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein a, b, c, h, i, j, r, s and t are each independently an integer between about 0 and about 3; wherein m and n are each independently an integer between about 0 and about 5;

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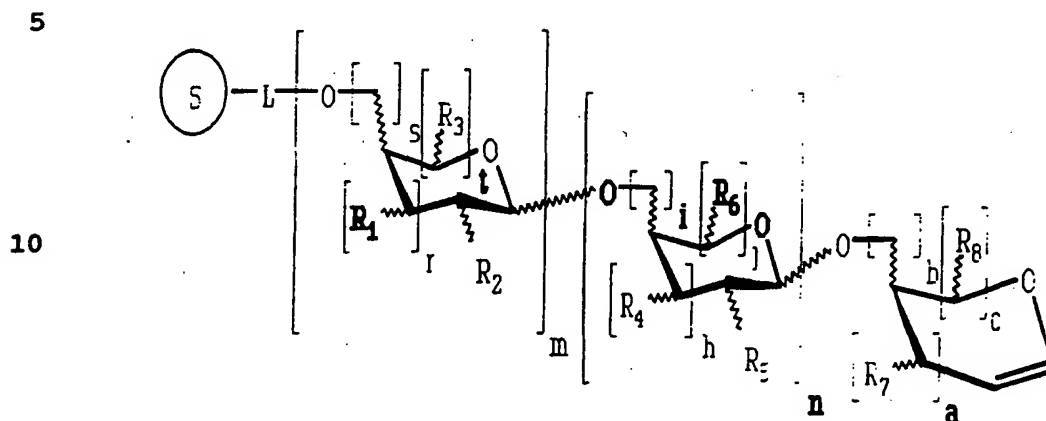
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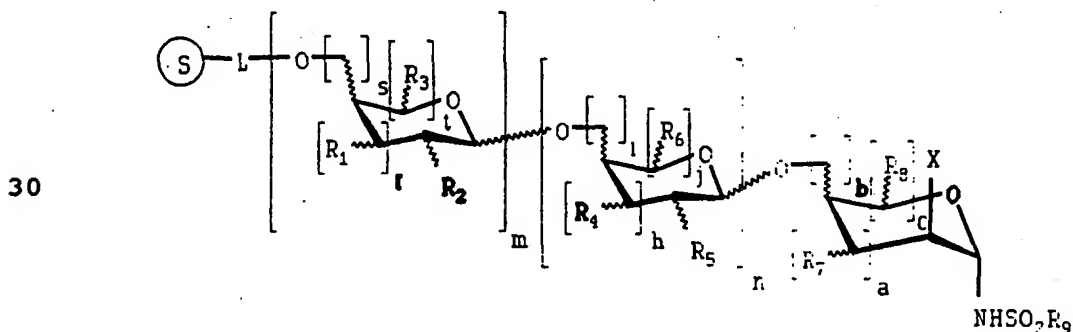
-56-

wherein q is an integer between about 1 and about 9; and wherein x , $x'(N)$, y and $y'(N)$ are each independently an integer between about 0 and about 25; which comprises:
 (a) halosulfonamidating a compound having the structure:



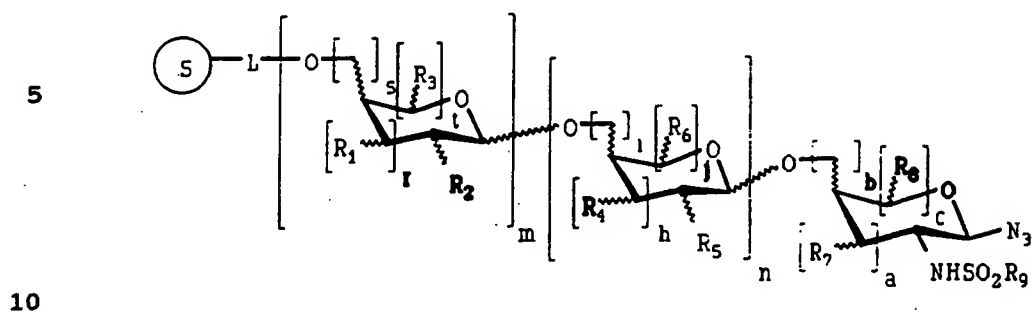
15 wherein L is a suitable linking moiety selected from the group consisting of SiR_2 , a substituted or unsubstituted linear or branched chain alkyl, arylalkyl, and aryl groups, where R is a linear or branched chain alkyl, alkoxy, arylalkyl, arylalkoxy or aryl group;
 20 wherein (S) is a polymeric solid-phase; with a compound

having the formula $\text{R}_9\text{SO}_2\text{NH}_2$, wherein R_9 is a substituted or unsubstituted, or a linear or branched chain alkyl, arylalkyl or aryl group under suitable conditions to form
 25 a compound having the structure:

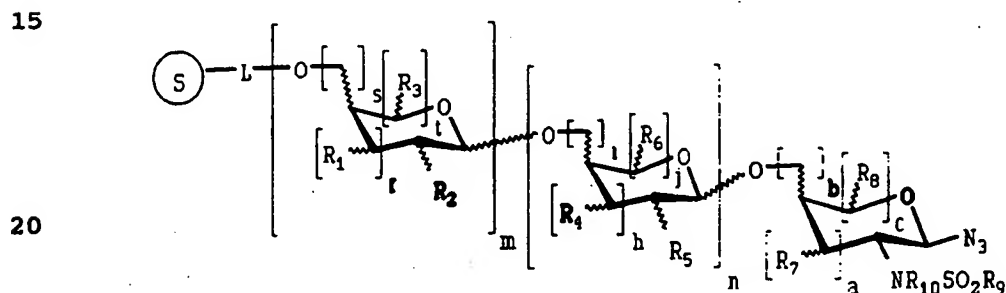


wherein X is selected from the group consisting of F , Cl , Br and I ; (b) reacting the compound formed in step (a)

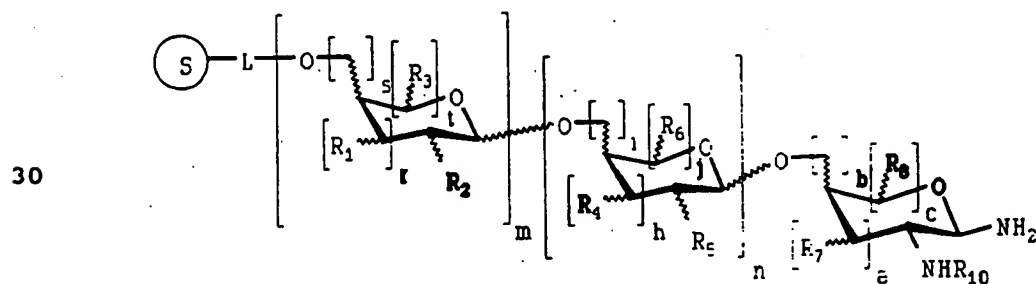
with an azide salt under suitable conditions to form a sulfonamide azide having the structure:



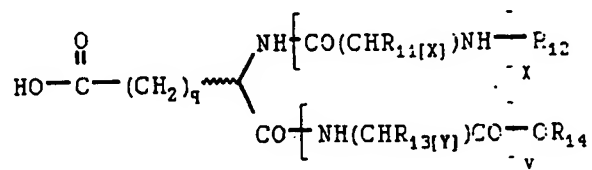
(c) acylating the sulfonamide azide formed in step (b) under suitable conditions to form an N-acylsulfonamide having the structure:



(d) reducing the N-acylsulfonamide formed in step (c) with a reducing agent under suitable conditions to form an amine N-acylamide having the structure:

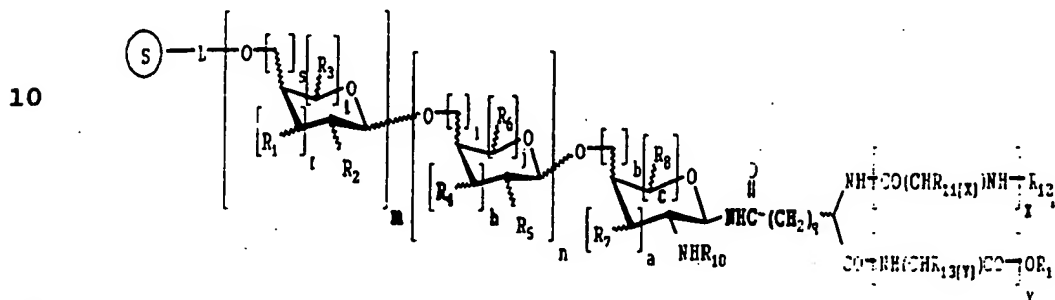


(e) coupling the amine N-acylamide with a suitably
35 protected acidic peptide having the structure:

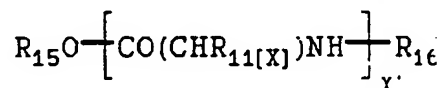


-58-

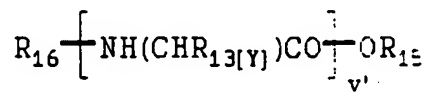
wherein R_{12} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; wherein R_{14} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein X is an integer between about 1 and x; under suitable conditions to form a protected glycopeptide having the structure:



(f) (i) selectively deprotecting the protected glycopeptide formed in step (e) under suitable conditions to form either an N- or C-deprotected glycopeptide; (ii) coupling the N- or C-deprotected glycopeptide respectively under suitable conditions with a protected amino acid or oligopeptide having the structure:



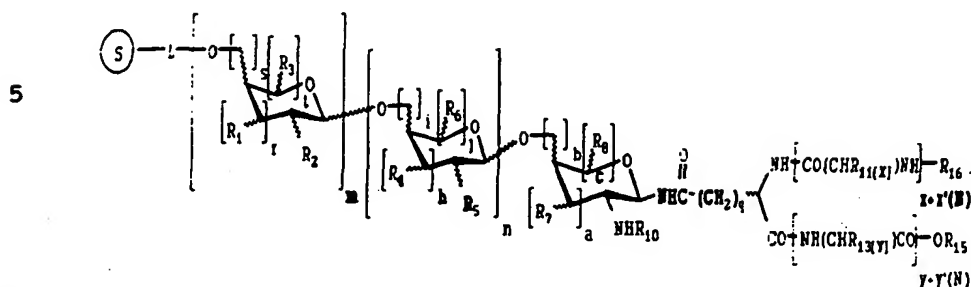
wherein R_{15} is H; and wherein R_{16} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; or having the structure:



wherein R_{16} is H; and wherein R_{15} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and (iii) optionally repeating iteratively

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steps (i) and (ii) N times to form a chain-extended glycopeptide having the structure:



and (g) cleaving and deprotecting the chain-extended glycopeptide under suitable conditions to form the glycopeptide.

15 In one embodiment, the present invention provides a process wherein b , i and s are each 1. In another embodiment, the present invention provides a process wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are OH.

20 In another embodiment, the present invention provides a process wherein X is I and R_9 is 10-anthracenyl.

In yet another embodiment, the present invention provides a process wherein R is isopropyl. In another embodiment, the present invention provides a process wherein the polymeric solid phase is selected from the group consisting of a polystyrene resin, silica gel, glass beads, an agarose resin and a polyacrylamide resin.

25 In one embodiment, the present invention provides a process wherein the polymeric solid phase is cross-linked polystyrene. In another embodiment, the present invention provides process wherein the solid phase is cross-linked with 1% divinyl benzene. In another embodiment, the present invention provides a process wherein a , c , h , j , r , t , m and n are each 1.

30 In another embodiment, the present invention provides a process wherein R_{12} is selected from the group consisting of t -butoxycarbonyl, t -amyloxycarbonyl, 9-fluorenylmethoxycarbonyl, benzyloxycarbonyl and biphenylisopropylloxycarbonyl. In yet another embodiment, the present invention provides a process wherein R_{14} is selected from the group consisting of methyl, ethyl, t -

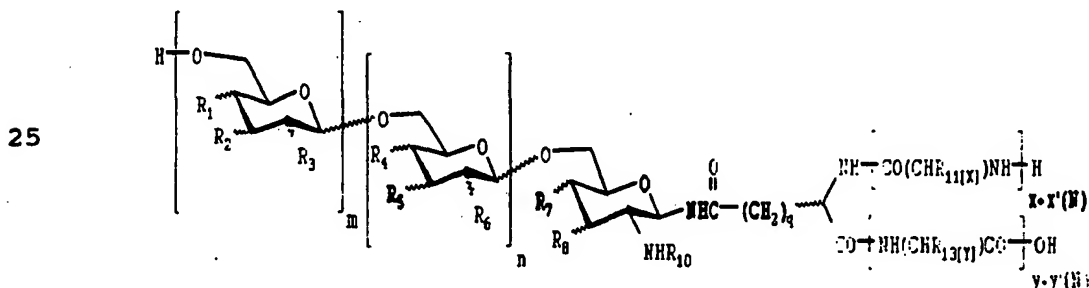
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butyl, benzyl, p-bromobenzyl, 2,4-dichlorobenzyl, α,α -dimethylbenzyl, trityl, phenacyl and benzhydryl.

Step (a) is preferably performed using 9-anthracene-sulfonamide, and ascorbic acid is the preferred agent for quenching the reaction. Step (b) is preferably performed using a tetraalkylammonium azide salt, most preferably where the alkyl moiety is n-butyl. Step (c) may be performed with a variety of reducing agents, but preferably using thiophenol and N,N-diisopropyl-N-ethylamine. The coupling and deprotecting steps (e) and (f) (i) and (ii) are carried out using methods of peptide synthesis known in the art (see, for example, M. Bodanszky, Principles of Peptide Synthesis, Springer-Verlag, 1984). A preferred N-protecting group is 2,2,2-trichloroethoxycarbonyl; a preferred C-protecting group is the allyl group. Cleavage and deprotection step (g) is carried out using HF-pyridine.

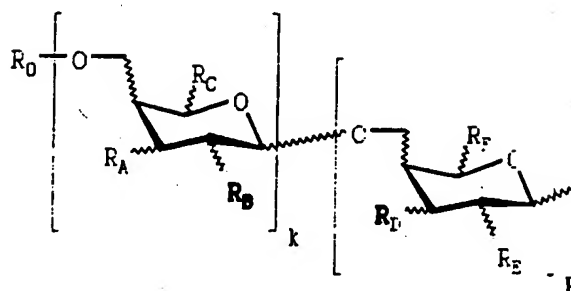
The present invention also provides a process of synthesizing a glycopeptide having the structure:



30 wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are each independently H, OH, OR^i , NH_2 , NHCOR^i , F, CH_2OH , CH_2OR^i , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R^i is H, CHO, CO_2R^{ii} , a linear or branched chain alkyl, arylalkyl or

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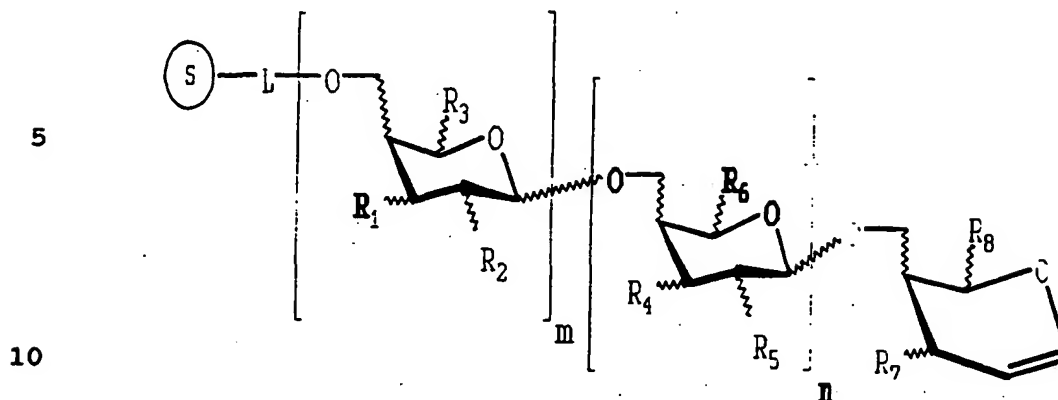
aryl group, or an oligosaccharide moiety having the structure:



wherein R_0 is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein k and p , are each independently 0, 1 or 2; wherein R_A , R_B , R_C , R_D , R_E and R_F are each independently H, OH, OR^{iii} , NH_2 , $NHCOR^{iii}$, F, CH_2OH , CH_2OR^{iii} , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, wherein R^{iii} is H, CHO, CO_2R^{iv} , a linear or branched chain alkyl, arylalkyl or aryl group, and wherein R^{ii} and R^{iv} are independently a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_{10} is a substituted or unsubstituted linear or branched chain acyl, arylacyl or aroyl group; wherein $R_{11}[X]$ represent X amino acid side-chains, where X is an integer from 1 to $x+x'(N)$, and denotes position from the N-terminus, and $x'(N)$ is a summation over N , where N is an integer from 1 to 10, and $R_{13}[Y]$ represent Y amino acid side-chains, where Y is an integer from 1 to $y+y'(N)$, and denotes position from the C-terminus, and $y'(N)$ is a summation over N , where N is an integer from 1 to 10, wherein each $R_{11}[X]$ and $R_{13}[Y]$ are independently the same or different, and are H, OH, a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein m and n are each independently an integer between about 0 and about 5; wherein q is an integer between about 1 and about 9; and wherein x , $x'(N)$, y and $y'(N)$ are each independently an integer between about 0 and about 25; which comprises:

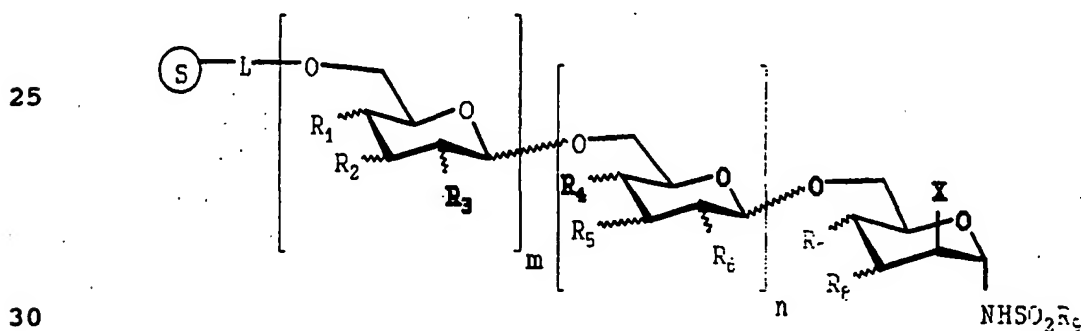
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(a) halosulfonamidating a compound having the structure:



wherein L is a suitable linking moiety selected from the group consisting of SiR_2 , a substituted or unsubstituted linear or branched chain alkyl, arylalkyl, and aryl groups, where R is a linear or branched chain alkyl, alkoxy, arylalkyl, arylalkoxy or aryl group; wherein (S) is a polymeric solid-phase; with a compound

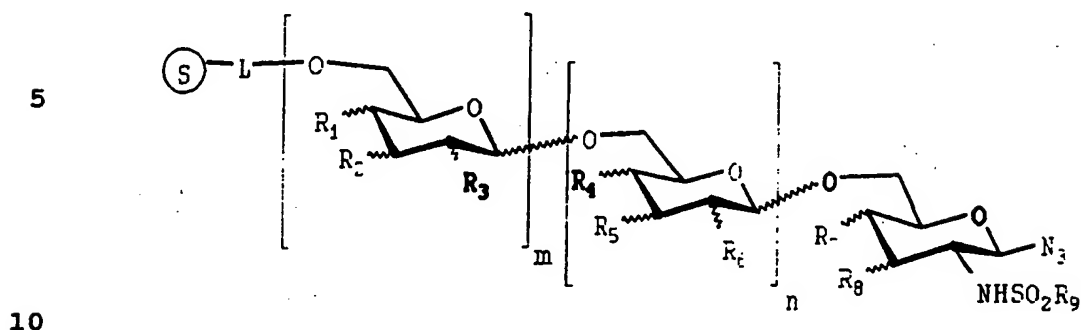
20 having the formula $\text{R}_9\text{SO}_2\text{NH}_2$, wherein R_9 is a substituted or unsubstituted, or a linear or branched chain alkyl, arylalkyl or aryl group under suitable conditions to form a compound having the structure:



wherein X is selected from the group consisting of F, Cl, Br and I; (b) reacting the compound formed in step (a)

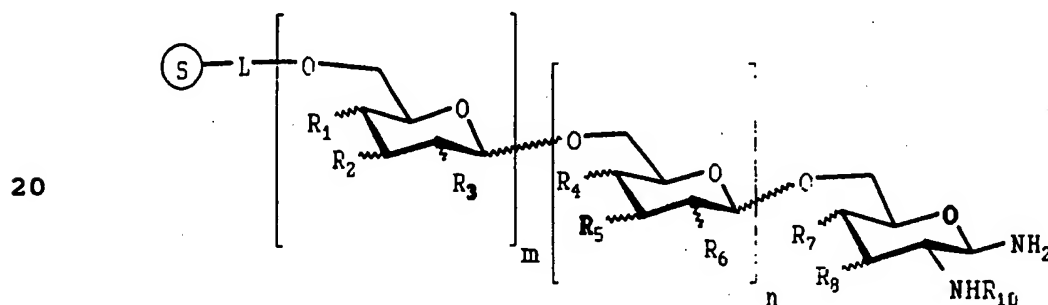
-63-

with an azide salt under suitable conditions to form a sulfonamide azide having the structure:



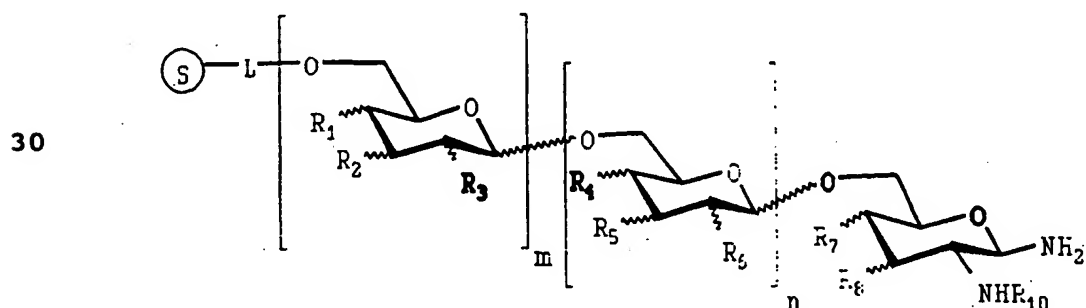
(c) acylating the sulfonamide azide formed in step (b) under suitable conditions to form an N-acylsulfonamide having the structure:

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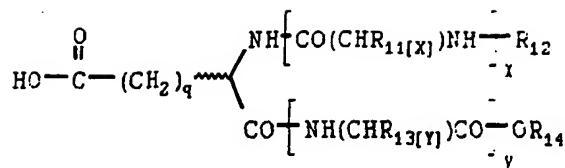
(d) reducing the N-acylsulfonamide formed in step (c) with a reducing agent under suitable conditions to form an amine N-acylamide having the structure:

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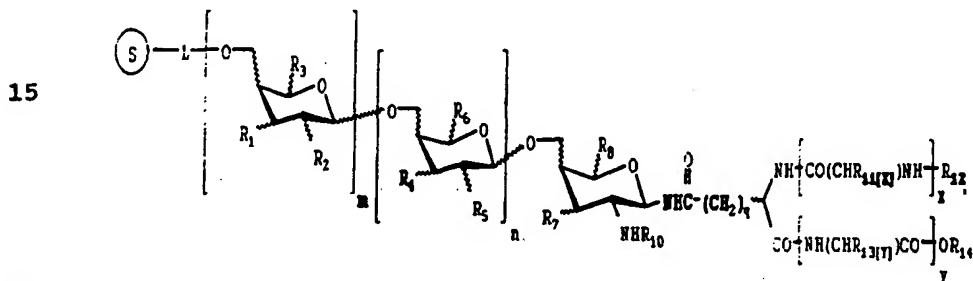
(e) coupling the amine N-acylamide with a suitably protected acidic peptide having the structure:

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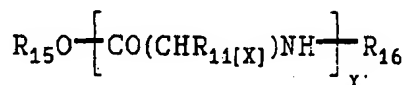
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wherein R_{12} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; wherein R_{14} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein X is an integer between about 1 and x ; under suitable conditions to form a protected glycopeptide having the structure:



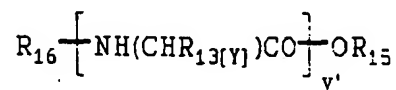
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(f) (i) selectively deprotecting the protected glycopeptide formed in step (e) under suitable conditions to form either an N- or C-deprotected glycopeptide; (ii) coupling the N- or C-deprotected glycopeptide respectively under suitable conditions with a protected amino acid or oligopeptide having the structure:



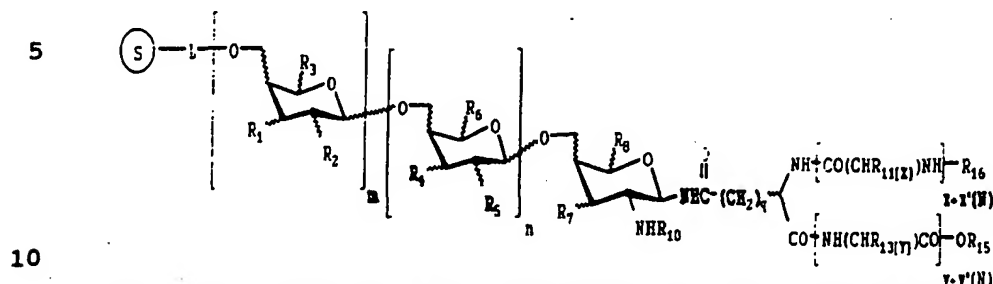
30 wherein R_{15} is H; and wherein R_{16} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; or having the structure:

35



40 wherein R_{16} is H; and wherein R_{15} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl

or aryl group; and (iii) optionally repeating iteratively steps (i) and (ii) N times to form a chain-extended glycopeptide having the structure:



and (g) cleaving and deprotecting the chain-extended glycopeptide under suitable conditions to form the glycopeptide. In one embodiment, the present invention provides a process wherein b, i and s are each 1. In another embodiment, the present invention provides a process wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are OH. In another embodiment, the present invention provides a process wherein X is I and R_9 is 10-anthracenyl. In yet another embodiment, the present invention provides a process wherein R is isopropyl. In another embodiment, the present invention provides a process wherein the polymeric solid phase is selected from the group consisting of a polystyrene resin, silica gel, glass beads, an agarose resin and a polyacrylamide resin. In one embodiment, the present invention provides a process wherein the polymeric solid phase is cross-linked polystyrene. In another embodiment, the present invention provides process wherein the solid phase is cross-linked with 1% divinyl benzene. In another embodiment, the present invention provides a process wherein a, c, h, j, r, t, m and n are each 1. In another embodiment, the present invention provides a process wherein R_{12} is selected from the group consisting of t-butyloxycarbonyl, t-amylloxycarbonyl, 9-fluorenylmethyloxycarbonyl, benzyloxycarbonyl and biphenylisopropylloxycarbonyl. In yet another embodiment, the present invention provides a process wherein R_{14} is selected from the group consisting of methyl, ethyl, t-butyl, benzyl, p-bromobenzyl, 2,4-dichlorobenzyl, α,α -dimethylbenzyl, trityl, phenacyl and benzhydryl.

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Experimental DetailsGeneral Procedures

5 All air- and moisture-sensitive reactions were performed in a flame-dried apparatus under an argon atmosphere unless otherwise noted. Air-sensitive liquids and solutions were transferred via syringe or canula. Wherever possible, reactions were monitored by thin-layer
10 chromatography (TLC). Gross solvent removal was performed in vacuum under aspirator vacuum on a Buchi rotary evaporator, and trace solvent was removed on a high vacuum pump at 0.1-0.5 mmHg.

Melting points (mp) were uncorrected and performed in
15 soft glass capillary tubes using an Electrothermal series IA9100 digital melting point apparatus.

Infrared spectra (IR) were recorded using a Perkin-Elmer 1600 series Fourier-Transform instrument. Samples were
20 prepared as neat films on NaCl plates unless otherwise noted. Absorption bands are reported in wavenumbers (cm^{-1}).

Only relevant, assignable bands are reported.

25 Proton nuclear magnetic resonance (^1H NMR) spectra were determined using a Bruker AMX-400 spectrometer at 400 MHz. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane (TMS; $\delta=0$ ppm) using residual CHCl_3 as a lock reference ($\delta=7.25$ ppm).
30 Multiplicities are abbreviated in the usual fashion: s=singlet; d=doublet; t=triplet; q=quartet; m=multiplet; br=broad.

Carbon nuclear magnetic resonance (^{13}C NMR) spectra were
35 performed on a Bruker AMX-400 spectrometer at 100 MHz with composite pulse decoupling. Samples were prepared as with ^1H NMR spectra, and chemical shifts are reported

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relative to TMS (0 ppm); residual CHCl_3 was used as an internal reference ($\delta=77.0$ ppm).

5 All high resolution mass spectral (HRMS) analyses were determined by electron impact ionization (EI) on a JEOL JMS-DX 303HF mass spectrometer with perfluorokerosene (PFK) as an internal standard. Low resolution mass spectra (MS) were determined by either electron impact ionization (EI) or chemical ionization (CI) using the
10 indicated carrier gas (ammonia or methane) on a Delsi-Nermag R-10-10 mass spectrometer. For gas chromatography/mass spectra (GCMS), a DB-5 fused capillary column (30 m, 0.25mm thickness) was used with helium as the carrier gas. Typical conditions used a
15 temperature program from 60-250°C at 40°C/min.

Thin layer chromatography (TLC) was performed using precoated glass plates (silica gel 60, 0.25 mm thickness). Visualization was done by illumination with
20 a 254 nm UV lamp, or by immersion in anisaldehyde stain (9.2 mL p-anisaldehyde in 3.5 mL acetic acid, 12.5 mL conc. sulfuric acid and 338 mL 95% ethanol (EtOH)) and heating to colorization.

25 Flash silica gel chromatography was carried out according to the standard protocol.

Unless otherwise noted, all solvents and reagents were commercial grade and were used as received, except as
30 indicated hereinbelow, where solvents were distilled under argon using the drying methods listed in parentheses: CH_2Cl_2 (CaH_2); benzene (CaH_2); THF (Na/ketyl); Et_2O (Na/ketyl); diisopropylamine (CaH_2).

35 Abbreviations

OTf triflate

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	TLC	thin layer chromatography
	EtOAc	ethyl acetate
	TIPS	triisopropylsilyl
	PMB	p-methoxybenzyl
5	Bn	benzyl
	Ac	acetate
	hex	hexane
	THF	tetrahydrofuran
	coll	collidine
10	LiHMDS	lithium hexamethyldisilazide
	DMF	N,N-dimethylformamide
	DMAP	2-dimethylaminopyridine
	DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
	TBAF	tetra-n-butylammonium fluoride
15	M.S.	molecular sieves
	r.t.	room temperature
	r.b.	round bottom flask

EXAMPLE 1

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Preparation of Polymer-Bound Glucal 18:

Polymer-bound galactal 7 (500 mg; S.J. Danishefsky, et al., *J. Am. Chem. Soc.* 1992, 8331) was placed in a 100 mL polymer flask and dried in vacuo. On cooling to 0°C under N₂, dry CH₂Cl₂ (20 mL) and freshly prepared Murray solution (30 mL; R.W. Murray and R. Jeyaraman, *J. Org. Chem.* 1985, 2847) was added. After stirring at 0°C for ~90 min., solubles were filtered using N₂ pressure. The oxidation procedure was repeated. The resulting epoxide of 7 kept on a vacuum line for ~3 h to dry. A solution of glucal 19 (1.0 g in 8 mL dry THF) was added, and the mixture was cooled to -23°C (dry ice-CCl₄). A solution of ZnCl₂ in THF (0.8 mL 1.0 M) was added. The mixture was slowly allowed to warm to r.t. (over ~2 h), and then stirred at r.t. overnight. The polymer-bound glucal 18

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was rinsed with 3 x 20 mL THF, and dried on a vacuum line.

Preparation of Polymer-Bound Tetrasaccharide 20:

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Polymer-bound glucal 18 and $\text{Sn}(\text{OTf})_2$ (0.80 g, 1.92 mmol) were combined and dried in vacuo. On cooling to 0°C under N_2 , a solution of fucosyl donor 10 (1.8 g, 4.1 mmol) in 20 mL dry THF with di-*t*-butylpyridine (1.7 mL, 7.57 mmol) was added. The mixture was allowed to warm slowly to r.t., and stirred overnight. The polymer was washed with 2 x 20 mL dry THF, 2 x 20 mL dry dioxane, 20 mL DMSO, and 2 x 20 mL THF. The resulting polymer-bound tetrasaccharide 20 was kept on a vacuum line to dry.

15

Preparation of Tetrasaccharide Glycal 21:

The polymer-bound tetrasaccharide 20 (50 mg) was stirred in 2 mL THF, and treated with 0.2 mL each of 1.0 M solutions of TBAF and AcOH in THF. The mixture was stirred at 40°C overnight. The polymer was washed with 3 x 5 mL THF. The combined rinsings were concentrated and column-chromatographed on silica (2:1 EtOAc:hex), providing tetrasaccharide glycal 21 as a colorless gum. Yield: 9.0 mg.

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EXAMPLE 2

Preparation of Diol 18':

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Galactal 7' (0.100 g, 0.304 mmol) in 5 mL dry CH_2Cl_2 at 0°C under a N_2 atmosphere was treated with 10 mL Murray solution (freshly prepared) and stirred at 0°C for 40 min. TLC (1:1 EtOAc:hex) showed no trace of 7'. Solvents were evaporated using a dry N_2 stream. The residual epoxide of 7' was kept on a vac. line -2h. To the epoxide under a N_2 atmosphere was added a solution of

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glucal derivative 3' (0.150 g, 0.496 mmol) in 3 mL dry THF. On cooling to -78°C , 1.0 M ZnCl_2 in Et_2O (0.50 mL, 0.50 mmol) was added. The mixture was allowed to slowly warm to r.t. (over ~2 h) and stirred overnight. TLC (1:1 EtOAc:hex) showed that the reaction was complete. Saturated aq. NaHCO_3 (20 mL) was added, and the mixture was then extracted with EtOAc (3 x 20 mL). The organic layer was dried over MgSO_4 . Column chromatography on silica (1:3 EtOAc:hex) afforded diol 18' as a colorless solid. Yield: 173 mg (89%). $[\alpha]_D^{23} - 9.8^{\circ}$ (c 1.0, CH_2Cl_2).

Preparation of Tetrasaccharide 22:

Diol 18' (86 mg, 0.133 mmol) and fucosyl donor 10 (0.290 g, 0.665 mmol) were azeotropically dried using benzene. The mixture was dissolved in 3 mL dry THF together with 0.65 mL di-*t*-butylpyridine and then added via canula to a flask containing $\text{Sn}(\text{OTf})_2$ (0.30 g, 0.72 mmol) and 4 Å MS (500 mg) at 0°C under N_2 atm. The mixture was stirred at 0°C ~7 h. TLC (1:3 EtOAc:hex) shows no trace of diol 18'. The mixture was partitioned between saturated aq. NaHCO_3 (100 mL) and EtOAc (2 x 100 mL). The organic layer was dried over MgSO_4 . The organic layer was filtered through silica using EtOAc to obtain crude material, which was then purified by chromatography on silica (1:9 EtOAc:hex) affording tetrasaccharide 22. Yield: 170 mg (86%).

Preparation of Iodosulfonamide 23:

Procedure 1.

Tetrasaccharide glycal 22 (120 mg, 81.1 mmol) and PhSO_2NH_2 (20 mg, 0.13 mmol) were azeotropically dried using benzene. Added (glove bag) 4 Å MS (0.2 g). After cooling to 0°C under N_2 , dry CH_2Cl_2 (1.0 mL) was added. The mixture was treated with a solution of $\text{I}(\text{coll})_2\text{ClO}_4$

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(prepared from 100 mg $\text{Ag}(\text{coll})_2\text{ClO}_4$, 5 mL collidine, and 60 mg I_2 in 1 mL dry CH_2Cl_2) via canula through a plug of flame-dried celite and 4 Å MS. The mixture was stirred at 0°C for 40 min. TLC (1:4 EtOAc:hex) showed
5 iodosulfonamide 23 as the major component. The mixture was filtered through celite, which was rinsed with Et_2O . The organic layer was extracted with saturated aq. $\text{Na}_2\text{S}_2\text{O}_3$, saturated aq. CuSO_4 , brine, and then dried over MgSO_4 . Column chromatography on silica (1:4 EtOAc:hex)
10 gave iodosulfonamide 23 as a colorless solid.
Yield: 115 mg (80%).

Procedure 2.

Tetrasaccharide glycal 22 (200 mg, 0.135 mmol), PhSO_2NH_2
15 (42 mg, 0.27 mmol), and 200 mg powdered 4 Å MS in 2.0 mL dry CH_2Cl_2 at 0°C under a N_2 atmosphere was treated with $\text{I}(\text{coll})_2\text{ClO}_4$ (prepared from 120 mg $\text{Ag}(\text{coll})_2\text{ClO}_4$ and 67 mg I_2 in 1 mL dry CH_2Cl_2). The mixture was stirred at 0°C (protected from light using foil) for 30 min. TLC (1:2
20 EtOAc:hex) showed mainly iodosulfonamide with some glycal.

After ~1 h more at 0°C, TLC showed no noticeable improvement. The mixture was filtered through celite, which was washed with Et_2O . After extracting with saturated aq.
25 $\text{Na}_2\text{S}_2\text{O}_3$, saturated aq. CuSO_4 , brine, the organics were dried over MgSO_4 . Column chromatography on silica (1:3 EtOAc:hex) gave 23 as a colorless solid.

Yield: 165 mg (69%). $[\alpha]_D^{23} = -85.7^\circ$ (c 1.0, CH_2Cl_2).

30

Preparation of Hexasaccharide 25:

Iodosulfonamide 23 (60 mg, 0.34 mmol) in a 35 mL r.b. was treated with 200 mg powdered 4 Å MS (glove bag). To this
35 flask under N_2 was added a solution of protected lactal 24 in THF (1.5 mL). On cooling the mixture to -78°C, a solution of AgBF_4 (40 mg, 0.206 mmol) was added in 0.25

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mL dry THF. The mixture was stirred and slowly warmed to r.t. overnight. The mixture was warmed to 45°C and stirred ~36 h. TLC showed only a trace of iodosulfonamide. Saturated aq. NH_4Cl (5 mL) was added, and the mixture was extracted with 3 x 10 mL EtOAc. The organic layer was dried over MgSO_4 . Column chromatography on silica (1:3 EtOAc:hex) afforded 25 as a colorless oil. Yield: 42 mg (55%).

$[\alpha]_D^{23} = -33.8^\circ$ (c 2.0, CH_2Cl_2)

Preparation of Hexasaccharide 25a:

Hexasaccharide 25 (55 mg, 24.4 mmol) in ~1.5 mL THF was treated at 0°C with TBAF (0.25 mL, 1.0 M solution in THF, 0.25 mmol), and stirred at r.t. overnight. TLC (1:9 MeOH: CH_2Cl_2) showed a 3:1 mixture of 25a vs. a less polar substance. Additional 1.0 M TBAF (0.10 mL) was added, and the mixture was stirred overnight at r.t. TLC showed that the reaction was complete. Solvents were removed using a N_2 stream. Column chromatography on silica (1:19 MeOH: CH_2Cl_2) afforded a ~1:2 mixture corresponding to two compounds which differ only in the presence or absence of a 3,4-cyclic carbonate group. Crude yield: 35 mg total weight for two products. The crude mixture was used as such for the next reaction.

Preparation of Peracetylated Hexasaccharide 26:

Hexasaccharide 25a (36 mg) in 0.25 mL dry THF was added via canula to ~8 mL bright blue Na/NH_3 solution at -78°C (dry ice bath) under N_2 atm. After removing the dry ice bath, the mixture was stirred in refluxing NH_3 (dry ice condenser) for 15 min. After adding 2 mL dry MeOH (slowly!), the resulting mixture was stirred while blowing off NH_3 with a N_2 stream. The MeOH solution was treated with Dowex 50 x 8 [H^+] until pH ~8-9, and then

-73-

filtered. The resin was washed with MeOH. The residue was concentrated and kept on a vacuum line to dry. Under a N₂ atmosphere, the residue was treated with 1 mL dry pyridine and 0.5 mL Ac₂O, and stirred at r.t. overnight. TLC (EtOAc) showed that hexasaccharide 26 is major component. Upon concentration, the residue was purified by column chromatography on silica (1:4 hex:EtOAc).

Preparation of Hexasaccharide 17:

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Hexasaccharide 26 (10.0 mg, 6.3 mmol) under N₂ at 0°C was treated with 0.5 mL dry CH₂Cl₂. Dioxirane solution (0.20 mL) was added, and the mixture was stirred at 0°C ~40 min. TLC (EtOAc) showed no trace of 26. Solvents were evaporated with a N₂ stream. The epoxide was dried on a vacuum line for ~2 h. The epoxide was treated under a N₂ atmosphere with 0.5 mL allyl alcohol (passed through basic alumina to dry) and 0.5 mL dry THF. On cooling to -78°C, 1.0 M ZnCl₂ (10 mL) in dry Et₂O was added. After warming slowly to r.t., the mixture was stirred overnight. Saturated aq. NaHCO₃ (5 mL) was added, and the mixture was extracted with 3 x 5 mL EtOAc. The combined organic layers were dried over MgSO₄, filtered and concentrated to an oil, which was dried on a vacuum line for ~2 h. The residue was treated to pyridine:Ac₂O (2:1, 1.5 mL) while stirring overnight. Solvents were removed, and the residue was purified by column chromatography on silica (1:4 hex:EtOAc), affording hexasaccharide 17 as a colorless solid. Yield: 5.5 mg.

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EXAMPLE 3

Synthesis of 3b:

3-O-(4-Methoxybenzyl)-D-galactal

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A suspension of D-galactal (2b) (3.70g, 25.3 mmol) and dibutyltin oxide (6.30g, 1.0 equiv) in dry benzene (150 mL) was heated to reflux for 2h with azeotropic removal

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of water. The reaction was cooled and treated with PMBCl (3.80 mL, 1.1 equiv) and tetrabutylammonium bromide (9.10g, 1.1 equiv) and refluxed for 4h. The reaction was filtered through silica column and eluted with EtOAc/hexanes (4:1). Fractions containing product were concentrated and the residue triturated in hexanes to give 4.50g (67%) of product as white crystalline solid.

mp (hexanes) 117-118 °C; $(\alpha)^{23}_D = -23.0^\circ$ (CHCl_3 , $c = 1.1$); IR (KBr) 3313 (br), 1645, 1513, 1228, 1082, 821 cm^{-1} ; ^1H -NMR (400 MHz, CDCl_3) δ 7.28 (2H, d, $J = 8.4$ Hz), 6.89 (2H, d, $J = 8.4$ Hz), 6.44 (1H, dd, $J = 6.4$ Hz), 4.70 (1H, dt, $J = 6.3, 1.9$ Hz), 4.59-4.52 (2H, ABq, $J = 11.4$ Hz), 4.20-4.18 (1H, m), 4.04-3.97 (1H, m), 3.90-3.82 (2H, m), 3.81 (3H, s), 2.73 (1H, d, $J = 3.1$ Hz, C4-OH), 2.54 (1H, dd, $J = 8.2, 4.2$ Hz, C6-OH); ^{13}C -NMR (100 MHz, CDCl_3) δ 159.46, 145.02, 142.05, 129.46, 113.95, 99.36, 76.12, 70.17, 70.14, 63.65, 62.74, 55.26; LRMS(NH_3) 284 ($\text{M} + \text{NH}_4$) $^+$, 266 (M) $^+$, 249.

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4,6-di-O-benzyl-3-O-(4-methoxybenzyl)-D-galactal (3b).

A solution of 3-O-(4-methoxybenzyl)-D-galactal (2.28 g, 8.56 mmol) and benzyl bromide (3.75 mL, 3.68 mol equiv; freshly passed through basic alumina) in DMF (30 mL) under N_2 at 0 °C was treated with NaH (1.37 g, 4.0 mol equiv) in two portions. The reaction was stirred 0.5h at 0 °C and 1h at rt. The reaction was carefully poured into 50 g of crushed ice, diluted to 100 mL with water, then extracted with EtOAc-hexanes (1:1, 100 mL x 3). Organic extracts were washed with water (100mL x 2), dried (Na_2SO_4) and concentrated. Flash chromatography with 15% EtOAc- hexanes gave 3.58 g (94%) of the title compound as a clear liquid.

$[\alpha]^{23}_D = -48.2^\circ$ (CHCl_3 , $c = 0.85$); IR (n at) 3030, 2867, 1645, 1613, 1513 1247, 1092, 821, 736 cm^{-1} ; ^1H -NMR (400 MHz, CDCl_3) δ 7.34-7.23(12H, m), 4.62 (1H, d, $J = 12.0$

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Hz), 4.59-4.51 (2H, ABq, $J = 11.7$ Hz), 4.50-4.39 (2H, ABq, $J = 11.9$ Hz) ^{13}C -NMR (100 MHz, CDCl_3) δ 159.04, 143.99, 138.30, 137.90, 130.43, 128.26, 128.20, 128.03, 127.77, 127.57, 127.56, 113.67, 100.00, 75.58, 73.28, 73.17, 71.13, 70.42, 70.28, 68.35, 55.15; LRMS (NH_3) 464 ($\text{M} + \text{NH}_4^+$, 100), 326 (18), 309 (48), 253 (17).

Synthesis of 4b:

A solution of galactal 3b (3.20g, 7.17 mmol) in dry CH_2Cl_2 under N_2 at 0°C was treated with dimethyldioxirane (0.09M, 80 mL) and stirred until all of the glycol was consumed (0.5-1h; TLC 30% EtOAc in hexanes). Volatiles were removed at 0°C with stream of dry N_2 . The residue was dissolved in 30 mL of dry THF under N_2 at 0°C and treated with TBAF (36 mL, stored over molecular sieves) then stirred at ambient temperature for 20h. The dark brown solution was filtered through a pad of silica (~4cm depth) and washed with EtOAc (200 mL). The filtrate was washed with water (200 mL x 3) and dried (MgSO_4) and concentrated. The residue was redissolved in 30% EtOAc-hexanes (50 mL) and filtered through short silica column (10 cm d x 4 cm h) and washed with the same solvent system (1L). The filtrate was concentrated to give 2.59g of fluorohydrin with >90% purity. The residue was dissolved in dry DMF (30 mL) under N_2 at 0°C and treated with benzyl bromide (958 μL , 1.5 equiv, freshly filtered through basic alumina), finally with NaH (322 mg, 60% dispersion, 1.5 equiv) and stirred for 30 min at 0°C and 30 min at rt. The reaction was quenched by pouring into 100g of ice, and extracted with 1:1 EtOAc-hexanes (150 mL x 2). The organic extracts were washed with water (150 mL x 2), dried (MgSO_4) and concentrated in vacuo. Flash chromatography with 10% EtOAc-hexanes gave 2.00g (49%) of the title compound as a yellowish liquid.

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$[\alpha]_D^{23} = +15.3^\circ$ (CHCl_3 , $c = 0.85$); IR (CHCl_3 film) 2916, 1612, 1513, 1248, 1103, 1056, 734 cm^{-1} ; ^1H -NMR (400 MHz,

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CDCl₃) δ 7.35-7.24 (17H, m), 6.84 (2H, d, J = 8.4 Hz), 5.15 (1H, dd, J = 53.2, 7.0 Hz), 4.92 (1H, d, J = 11.6 Hz), 4.48-4.74 (2H, ABq, J = 11.8 Hz), 3.96-3.89 (1H, m), 3.86 (1H, br s), (3H, s), 3.65-3.56 (3H, m), 3.51 (1H, dd, J = 9.8, 2.8 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 159.22, 138.33, 138.11, 137.62, 130.16, 129.19, 128.40, 128.29, 128.21, 128.04 (2C), 127.90, 127.81, 127.69, 127.59, 113.77, 110.20 (d, J = 214 Hz), 80.60 (d, J = 11.3 Hz), 79.00 (d, J = 20.5 Hz), 74.92, 74.52, 73.59 (d, J = 5.0 Hz), 73.54, 72.99, 72.70, 68.34, 55.20; LRMS (NH₃) 454 (M + NH₄⁺, 100).

Synthesis of 6b:

A solution of TIPS-carbonate galactal 5b (Danishefsky, S.J.; Behar, V.; Randolph, J.T.; Lloyd, K., *J. Am. Chem. Soc.*, 1995, 0000) (4.28g, 5.62 mmol) in THF (25mL)-MeOH (5 mL) was treated with TBAF solution (1.0M, 6.75 mL, 1.2 equiv). After 6h, additional TBAF (4mL) was added and stirred additional 3h. The reaction was concentrated and directly chromatographed with 4:1 EtOAc-hexanes to obtain 2.20g of the triol. Remaining mixtures of cyclic carbonate and mixed carbonate was hydrolysed in MeOH with MeONa (1.0 mL, 25 wt%) and purified chromatographically. Total yield was 3.02g (93%). This material was directly used for the dibenzylation step.

¹H-NMR (400 MHz, CDCl₃) δ 7.35-7.24 (15H, m), 6.43 (1H, d, J = 6.3 Hz), 4.87 (1H, dd, J = 6.3, 3.4 Hz), 4.84 (1H, d, J = 11.4 Hz), 4.63 (2H, apparent s), 4.61 (1H, d, J = 11.4 Hz), 4.53-4.47 (3H, m), 4.19-4.16 (3H, m), 3.87-3.84 (2H, m), 3.78-3.66 (3H, m), 3.46 (2H, apparent d, J = 4.6 Hz), 3.29 (1H, t, J = 5.5 Hz), 3.08 (1H, br), 2.73 (2H, br); ¹³C-NMR (100 MHz, CDCl₃) δ 144.70, 138.41, 138.22, 137.83, 128.45, 128.33 (2C), 128.12, 127.84, 127.73, 127.64, 127.57, 102.28, 99.74, 78.99, 76.03, 74.64, 74.07, 73.24 (2C), 73.17, 72.64, 70.20, 69.10, 67.79, 62.15.

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A mixture of triol glycal from above (2.95g, 5.1 mmol), dibutyltin oxide (1.33g, 1.05 equiv) and bistributyltin oxide (1.69 mL, 0.65 equiv) in dry benzene (50 mL) under N₂ was refluxed for 5h with azeotropic removal of water. 5 The reaction was cooled below boiling and treated with benzyl bromide (2.43 mL, 4.0 mol equiv) and tetrabutylammonium bromide (3.29g, 2.0 equiv). 10 mL of benzene was distilled off and the reaction refluxed for 16h. The reaction was directly loaded on silica column and eluted 10 with 15-20% EtOAc-hexanes to give 3.48g (90%) of product 6b as a clear oil.

$[\alpha]_D^{23} = -3.3^0$ (CHCl₃, c = 0.87); IR (CHCl₃ film) 2867, 1652 1454, 1364, 1097, 736 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.35-7.21 (25H, m), 6.45 (1H, d, J = 6.2 Hz), 4.88 (1H, dd, J = 6.2, 3.9Hz), 4.83 (1H, d, J = 10.9 Hz), 4.69 (2H, apparent s), 4.68 (1H, d, J=10.9Hz), 4.59 (2H, apparent s), 4.55 (1H, d, J = 7.8 Hz), 4.49 (2H, apparent s), 4.47 (2H, apparent s), 4.29 (1H, dd, J = 9.6, 5.8 Hz), 4.18 20 (1H, t, J = 4.4 Hz), 4.13 (1H, m), 3.99 (1H, br s), 3.85 (1H, dd, J = 10.6, 6.4 Hz), 3.75-3.60 (4H, m), 3.47-3.41 (2H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 144.43, 138.64, 138.42, 137.99, 137.84, 137.80, 128.40, 128.34, 128.26, 128.23, 128.18, 128.15, 127.82, 127.75, 127.69, 127.67, 25 127.65, 127.55, 127.51, 127.46, 127.31102.56, 99.56, 80.57, 78.69, 75.72, 75.10, 73.57, 73.32, 72.94, 72.28, 71.94, 70.12, 68.90, 67.85, 66.62; LRMS (NH₃) 776 (M + NH₄⁺, 100).

30 Synthesis of 7b:

Lactal 6b (1.32g, 1.74 mmol, 1.0 equiv) and fluoro sugar 4b (1.49g, 2.60 mmol, 1.5 equiv) were combined in ether and concentrated. The mixture were dried by evaporation in dry benzene (25 mL x 2), in vacuum for 2h then treated 35 with di-t-butylpyridine (389 uL, 1.0 equiv) in glove bag and dissolved in dry ether (18 mL) under nitrogen atm sphere. In a separate 50 mL flask was placed 4A M.S.

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(4.0g) then flame-dried under vacuum, cooled to room temperature. Anhydrous silver perchlorate (360 mg, 1.0 equiv) and SnCl_2 (329 mg, 1.0 equiv) were added in glove bag and flushed with nitrogen. The salt mixture was placed in water bath and sugar solution was introduced via double tipped needle and the mixture sonicated for 2 min. The reaction was wrapped with aluminum foil and stirred for 45h at rt. The filtrate (200 mL) was washed with dil NaHCO_3 (100 mL x 2), dried (MgSO_4) and concentrated. Flash chromatography with 15-20 % EtOAc/hexanes yielded trisaccharides (1.107g, 49%) and impure lactal. The trisaccharide portion was rechromatographed with 2% ether-methylene chloride to give 879 mg (39%) of the desired α -product and 195 mg (8.6%) of β -product. The impure lactal fraction was rechromatographed with 3-4% ether-methylene chloride to give 479 mg (36%) of clean lactal. 77% of coupling (61% α -product) yield based on recovered starting material.

[α] $^{23}_D$ = +41.8 0 (CHCl_3 , c = 1.8); IR (CHCl_3 film) 2867, 1648, 1513, 1496, 1453, 1364, 1248, 1097, 735 cm^{-1} ; ^1H -NMR (400 MHz, CDCl_3) δ 7.33-7.12 (42H, m) 6.83 (2H, d, J = 8.4 Hz), 6.45 (1H, d, J = 6.0 Hz), 5.03 (1H, d, J = 2.3 Hz), 4.91-4.76 (6H, m), 4.68-4.40 (12H, m), 4.23-3.97 (11H, m), 3.86-3.82 (1H, dd, J = 2.3 Hz), 3.76 (3H, s), 3.69-3.64 (2H, m), 3.53 (1H, t, J = 8.7 Hz), 3.47-3.43 (1H, m), 3.40-3.36 (1H, m), 3.34-3.31 (1H, dd, J = 9.9, 2.8 Hz), 3.22 (1H, dd, J = 8.3, 4.8 Hz); ^{13}C -NMR (100 MHz, CDCl_3) δ 158.93, 144.59, 138.98, 138.84, 138.78, 138.64, 138.58, 138.06, 138.02 (2C), 130.82, 129.04, 128.33, 128.24, 128.21, 128.15, 128.08, 128.05, 127.83, 127.81, 127.72, 127.64, 127.58, 127.55, 127.50, 127.44, 127.41, 127.36, 127.33, 127.31, 113.65, 103.02, 100.39, 100.01, 80.93, 78.93, 78.70, 76.53, 76.11, 75.14, 74.84, 74.79, 74.35, 73.91, 73.59, 73.36, 73.15, 73.10, 72.98, 72.15, 72.10, 71.99, 70.55, 69.25, 67.92 (2C), 67.69, 55.19.

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Synthesis of 8b:

A solution of PMB-trisaccharide (37 mg, 0.028 mmol) in CH_2Cl_2 (1 mL) at 0°C . The reaction was directly loaded on silica column and eluted with 20% EtOAc-hexanes to give
5 28 mg (84%) of desired product.

$[\alpha]_D^{23} = +45.6^\circ$ (CHCl_3 , $c = 1.78$); IR (CHCl_3 film) 2866, 1648, 1496, 1453, 1248, 1097, 735 cm^{-1} ; $^1\text{H-NMR}$ (400 MHz, CDCl_3) δ 7.36-7.15 (40H, M), 6.43 (1H, d, $J = 6.2$ Hz),
10 5.09 (1H, d, $J = 3.3$ Hz), 4.85 (1H, dd, $J = 6.2, 3.6$ Hz), 4.83-4.65 (5H, m), 4.61-4.41 (9H, m), 4.29-4.08 (8H, m), 4.02 (1H, d, $J = 2.6$ Hz), 3.97 (1H, d, $J = 2.2$ Hz), 3.93 (1H, t, $J = 8.4$ Hz), 3.86-3.78 (2H, m), 3.67-3.61 (2H, m), 3.53 (1H, dd, $J = 8.5, 4.8$ Hz); $^{13}\text{C-NMR}$ (100 MHz, CDCl_3) δ 144.38, 138.78, 138.62, 138.47, (2C), 138.20,
15 138.00, 137.88, (2C), 128.31, 128.29, 128.23, 128.19, 128.16, 128.05, 127.88, 127.83, 127.62, 127.57, 127.49, 127.45, 127.43, 127.41, 127.37, 127.32, 127.23, 102.68, 99.89, 99.34, 80.82, 78.72, 77.49, 77.10, 75.88, 75.13,
20 75.03, 74.23, 73.62, 73.05, 73.01, (3C), 72.62, 72.19 (2C), 70.46, 69.66, 68.92, 67.85, 67.74, 67.54.

Synthesis of 11b:

Glycal 9b (4.32 g, 3.14 mmol) was dissolved in CH_2Cl_2 (20
25 ml) and cooled to 0°C . It was then treated with dimethyldioxarane (219 ml, 3.14 mmol) at 0°C . The epoxidation finished within 1 h and then the reaction mixture was concentrated to dryness using dry N_2 stream. The residue was further azeotroped once with benzene (20
30 ml) and put on a vacuum line for 30 min at 0°C before being dissolved in THF (60 ml) and cooled to -78°C . Into the above solution was added, via canula, azeotropically dried galactal 10b (3.32 g, 10.95 mmol, 20 ml THF) and followed by ZnCl_2 (26.3 ml, 1.0 M in ether). The reaction
35 mixture was warmed up to room temperature and stirred overnight. After treatment with sat'd aq. Na_2CO_3 (40 ml), the reaction mixture was concentrated and extracted with

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ether (500 ml). The organic phase was washed with sat'd aq. NaCl, dried (MgSO₄) and concentrated. The crude product was purified by silica gel chromatography (1:4 EtOAc-hexanes) to give 6.20 g of 11b as a white foam (87.4%).

IR (CHCl₃ film) ν cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 6.45 (1H, dd, J = 6.4, 1.6 Hz), 4.85 (1H, dd, J = 6.4, 2.0 Hz), 4.72-4.68 (2H, m), 4.65 (1H, d, J = 7.2 Hz), 4.55 (1H, m), 4.21 (1H, m), 4.08 (1H, dd, J = 9.6, 5.6 Hz), 3.96-3.82 (6H, m), 3.33 (1H, d, J = 3.2 Hz, OH), 3.27 (1H, d, J = 2.8 Hz, OH), 1.16-1.04 (42H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 154.45, 145.75, 99.14, 98.27, 77.83, 76.59, 74.27, 72.04, 71.62, 70.86, 64.52, 62.57, 61.60, 17.84, 11.78, 11.77. ; LRMS (NH₃) 664 (M + NH₄⁺, 100), 647 (M + 1⁺, 5), 422 (21), 380 (25).

Synthesis of 13b:

Disaccharide 11b (2.64 g, 4.08 mmol) was azeotropically dried three times (3 x 10 ml) together with fluoro-fucose 12b (1.64 g, 3.77 mmol) and molecular sieves (4 A, 4.0 g) in THF (20 ml) with 2,6-di-tert-butylpyridine. The solution was added via canula to a flask containing AgClO₄ (1.56 g, 7.54 mmol), SnCl₂ (1.43 g, 7.54 mmol) and molecular sieves (4 A, 4.0 g) in THF (15 ml) at -40°C. The reaction mixture was stirred 30 min at -40°C and then 34 h at 5°C until the disappearance of fluoro-fucose. After treatment with sat'd aq. NaHCO₃ (40 ml) at 5°C, the mixture was extracted with EtOAc (700 ml). The organic phase was washed with sat'd NaCl, dried (MgSO₄) and concentrated. The crude product was purified by silica gel chromatography to give 1.93 g of the desired trisaccharide glycal 13b (48%, based on fluoro-fucose used) and 500 mg of the recovered disaccharide with only a trace of the other monofucosyl product.

EXAMPLE 4

Synthesis of 15b:

5 An azeotropically dried mixture of the trisaccharide glycal 13b (1.11 g, 1.05 mmol) and benzensulphonamide (0.82 g, 5.24 mmol) was dissolved in the THF (20 ml) together with molecular sieves (4 A, 2.6 g). The mixture was cooled to -40°C and then was added, via canula, a solution of I(sym-coll)₂COI₄ prepared *in situ* by
10 stirring I₂ (0.54 g, 2.09 mmol) with Ag(sym-coll)₂COI₄ (0.986 g, 2.29 mmol) in THF (20 ml) at room temperature for about 30 min until the disappearance of the brown color of I₂. The mixture was warmed up to 0°C within 1 h and stirred for another 1 h. After quenching with sat'd
15 aq. Na₂S₂O₃, the mixture was filtrate and extracted with EtOAc (3 x 100 ml). The combined organic phase was washed with sat'd aq. CuSO₄ (100 ml), sat'd NaCl (100 ml x 2) and dried (Na₂SO₄). After concentration, the crude product was purified by silica gel chromatography (1:4
20 EtOAc-hexanes) to give 981 mg of a colorless oil as a 21 mixture of the desired α -trans-diaxial iodosulphonamide and its *cis* isomer. The iodosulphonamide mixture was then added with stirring into a flask containing ethanthiol (226.3 mg, 3.64 mmol) and lithium
25 hexamethydisilylazide (1.46 ml, 1.46 mmol) in DMF (10 ml) at -40°C . The reaction mixture was stirred at -40°C overnight, and then quenched with sat'd aq. NaHCO₃ and extracted with ether (3 x 100 ml). The combined organic phase was washed with sat'd aq. NaCl and dried (Na₂SO₄).
30 After concentration, the crude product was purified by silica gel chromatography (3:97 EtOAc-CHCl₂) to yield 438 mg of 15b (33%) and 333 mg of the intact *cis* iodosulphonamide.

35 Synthesis of 16b:

A mixture of acceptor trisaccharide 8b (92 mg, 0.077 mmol, 1.0 equiv), thioglycoside 15b (198 mg, 2.0 equiv)

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and freshly activated 4Å-MS (560 mg) under N₂ at rt was suspended in CH₂Cl₂-Et₂O (1:2, 3.9 mL) and stirred for 10 min. The reaction was cooled to 0°C, then treated with methyl triflate (52.4 uL, 6.0 equiv). The reaction was stirred for 4.5h at 0°C and 1.5h while warming to 15°C. The reaction was quenched with TEA(1.0 mL), filtered through a pad of silica and rinsed with Et₂O. The filtrate (70 mL) was washed with sat'd NaHCO₃ (50 mL x 2), dried (Na₂SO₄) and concentrated. The crude product was purified by HPLC (17% EtOAc in hexanes, 15 mL/min, 260 nm UV dection) to give 158 mg (85%) of the desired product and 27.7 mg of α-linked byproduct (ca 55% purity).

Retention time=22 min; $[\alpha]^{23}_D = -13.3^0$ (CHCl₃, c = 1.4); IR (CHCl₃ film) 2940, 2865, 1792, 1652, 1454, 1161, 1101, 734 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.8 (2H, m), 7.38-7.06 (58H, m), 6.43 (1H, d, J = 6.1 Hz), 5.15 (1H, br s), 5.07 (1H, d, J = 3.6 Hz), 5.03 (1H, d, J = 3.6 Hz), 4.99 (1H, d, J = 11.6 Hz), 4.89-4.61 (12H, m), 4.54-4.46 (4H, m), 4.42 (2H, app s), 4.38 (1H, d, J = 11.9 Hz), 4.34-4.26 (3H, m), 4.21-4.18 (4H, m), 4.13-4.03 (7H, m), 3.98-3.76 (14H, m), 3.70-3.61 (4H, m), 3.46-3.27 (7H, m), 2.84 (1H, OH), 1.16 (3H, d, J = 6.4 Hz), 1.13-1.02 (42H, m); ¹³C-NMR (100 MHz, CDCl₃) δ 155.35, 144.55, 140.78, 138.99, 138.75, 138.68, 138.54, 138.43, 138.13, 138.03, 137.94, 137.82, 132.31, 128.81, 128.52, 128.38, 128.36, 128.27, 128.24, 128.20, 128.16, 128.02, 127.93, 127.72, 127.66, 127.58, 127.48, 127.43, 127.37, 127.20, 103.41, 102.75, 99.79, 99.55, 98.29, 97.76, 80.49, 80.39, 79.09, 78.91, 78.25, 77.68, 76.51, 75.88, 75.09, 74.99, 74.91, 74.73, 74.15, 74.02, 73.92, 73.52, 73.19, 73.10, 72.94, 72.67, 72.25, 72.07, 71.76, 71.56, 71.33, 70.33, 69.45, 69.32, 68.48, 68.08, 67.86, 67.75, 61.97, 61.60, 56.14, 17.99, 17.96, 17.95, 17.92, 16.75, 11.86; HRMS (FAB) calcd for C₁₃₈H₁₆₉NO₃₀SSi₂Na (M + Na) 2432.0920, found 2432.0970.

Synthesis f 19b:

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A solution of hexasaccharide glycal 16b (85 mg, 0.035 mmol) in THF (6mL) under N₂ at rt was treated with TBAF (1.0 M, 353 uL, 10 equiv). After 38h at rt, the reaction was concentrated to ca 1 mL, then dissolved in EtOAc (60 mL), washed with water (30 mL x 2), dried (Na₂SO₄) and concentrated. Flash chromatography with 4% MeOH in CH₂Cl₂ gave 70.0 mg (98%) of the desilyl-decarbonated product.

$[\alpha]_D^{23} = 1.8^0$ (CHCl₃ film) 2868, 1652, 1455, 1157, 1094, 735 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 7.80 (2H, d, J = 7.4 Hz), 7.47 (2H, d, J = 7.2 Hz), 7.37-6.95 (56H, m), 6.45 (1H, d, J = 6.3 Hz), 5.86 (1H, br s), 5.35 (1H, d, J = 11.6Hz), 5.30 (1H, D, J = 2.8 Hz), 4.95 (1H, d, J = 11.3 Hz), 4.89 (1H, d, J = 3.5 Hz), 4.8644.67 (9H, m), 4.54-4.39 (9H, m), 4.34 (1H, dd, J = 10.4, 2.8 Hz), 4.26-4.06 (9H, m), 3.98-3.45 (23H, m), 3.41 (1H, d, J = 10.0 Hz), 3.29-3.20 (5H, m), 0.73 (3H, d, J = 6.3 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 144.87, 142.49, 139.49, 139.11, 138.87, 138.63, 138.54, 138.37, 138.00, 137.98, 137.97, 137.18, 131.64, 128.74, 128.52, 128.43, 128.33, 128.28, 128.25, 128.21, 128.02, 127.99, 127.97, 127.80, 127.074, 127.67, 127.63, 127.61, 127.54, 127.53, 127.50, 127.44, 127.33, 127.31, 127.02, 126.86, 103.39, 102.78, 100.75, 100.09, 99.80, 99.75, 81.42, 80.64, 78.98, 78.86, 77.82, 77.40, 77.26, 76.26, 75.16, 75.09, 75.07, 74.95, 74.69, 74.30, 73.58, 73.17, 73.11, 72.71, 72.67, 72.65, 72.55, 72.36, 72.18, 69.65, 69.53, 68.54, 68.18, 68.08, 67.85, 67.79, 67.21, 54.95, 16.60.

To liquid ammonia (ca 8 mL) under N₂ at -78°C was added metallic sodium (95 mg) and stirred for 2 min. To the blue solution was added a solution of the hexasaccharide glycal above (70 gm, 33.8 umol) in dry THF(2mL). After 45 min at -78°C, the reaction was quenched with absolute methanol (4 mL). Most of ammonia was removed with stream of nitrogen (final volume was ca 4 mL) and the reaction diluted with methanol to ca 10 mL. To the solution was

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added Dowex 50-X8 (890 mg, washed and dried) and stirred for 5 min. The solution was filterate and rinsed with methanol, finally with ammoniacal methanol (5 mL), and the filterate was concentrated in vacuo. The residue and DMAP (2.4 mg) were placed under N₂ and suspended in DMF (1.0 mL), THF (1.0 mL) and TEA (1.0 mL), then treated with Ac₂O (0.3 mL). After 20h (TLC analysis with EtOAc), the reaction was poured into water (40 mL), and extracted with EtOAc (40 mL x 2), washed with dil NaHCO₃ (30 mL), with water (30 mL), dried (Na₂SO₄) and concentrated. Flash chromatography with 80% EtOAc in CH₂Cl₂ gave 52.0 mg (93%) of product as white foam.

mp 132-134°C; $[\alpha]_D^{23} = +4.7^\circ$ (CHCl₃, c = 1.4); IR (CHCl₃ film) 1742, 1652, 1371, 1227, 1069 cm⁻¹; ¹H-NMR (400 MHz, CDCl₃) δ 6.68 (1H, d, J = 6.8 Hz), 6.42 (1H, d, J = 6.0 Hz), 5.58 (1H, d, J = 3.2 Hz), 5.47 (1H, d, J = 3.4 Hz), 5.40-5.37 (2H, m), 5.29 (1H, dd, J = 10.9, 3.1 Hz), 5.25-5.15 (5H, m) 5.06 (1H, dd, J = 11.2, 3.3 Hz), 5.02 (1H, d, J = 3.6 Hz), 4.99-4.92 (2H, m), 4.84-4.81 (2H, m), 4.67 (1H, d, J = 7.8 Hz), 4.56-4.51 (2H, m), 4.45-4.38 (3H, m), 4.29 (1H, dd, J = 10.6, 3.4 Hz), 4.22-3.95 (13H, m), 3.90-3.77 (3H, m), 2.19-1.92 (51H, m), 1.15 (3H, d, J = 6.4 Hz); ¹³C-NMR (100 MHz, CDCl₃) δ 172.40, 171.45, 170.84, 170.54, 170.52, 170.48, 170.45, 170.40, 170.39, 170.34, 170.23, 169.99, 169.82, 169.74, 169.36, 169.00, 145.43, 102.01, 101.17, 98.83, (2C), 98.45, 94.24, 75.65, 74.95, 73.98, 73.64, 73.49, 72.32, 71.84, 71.53, 71.44, 70.81, 70.74, 70.66, 70.12, 69.77, 68.97, 68.71, 68.67, 68.02, 67.97, 67.88, 67.60, 67.35, 64.43, 61.88, 61.81, 61.42, 61.29, 61.04, 56.18, 23.06, 21.02, 20.81, 20.76, 20.68, 20.64, 20.62, 20.58, 20.57, 20.55, 20.49, 20.43, 15.88.

Peracetyl hexasaccharide glycal above (52 mg) was divided into two portions (22 mg and 30 mg). A solution of hexasaccharide glycal (22.0 mg, 13.4 μmol) in dry CH₂Cl₂

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(2 mL) under N₂ at 0°C, then treated with allyl alcohol (5 mL). The mixture was stirred for 15h at room temperature. Excess allyl alcohol was removed in vacuo. The other batch (30 mg) was treated similarly. The crude products were combined and chromatographed with 85% EtOAc-CH₂Cl₂ to give 35.8 mg (66%) of less polar product and 15.7 mg (29%) of more polar product. A 33.2 mg (19 umol) of the less polar material under N₂ was dissolved in absolute MeOH (14 mL) and treated with MeONa solution in methanol (165 uL, 25% by weight). After 6h, the reaction was neutralized with Dowex 50-X8 (200 mg, washed and dried), filtered and concentrated to give quantitative yield of the title compound 19b.

mp 204-206⁰(dec); $[\alpha]_D^{23} = +5.5^0$ (MeOH, c = 0.67); IR (MeOH film) 3356 (br), 2923, 1658, 1374, 1071 cm⁻¹; ¹H-NMR (400 MHz, CD₃OD) δ 5.99-5.93 (1H, m), 5.24 (1H, d, J = 3.8 Hz), 5.18-5.14 (1H, m), 4.93 (1H, d, J = 3.9 Hz), 4.56-4.54 (2H, m), 4.42-4.06 (10H, m), 3.99 (1H, s), 3.91-3.47 (26H, m), 3.41-3.37 (1H, m), 3.27 (1H, t, J = 8.8 Hz), 2.01 (3H, s), 1.24 (3H, d, J = 6.5 Hz); ¹³C-NMR (100 MHz, CD₃OD, ref = δ49.05) δ 174.55, 135.73, 117.57, 105.48, 105.42, 103.94, 103.26, 102.79, 101.08, 81.21, 80.67, 80.05, 79.20, 78.09, 76.79, 76.56, 76.48, 76.44, 76.41, 75.54, 74.86, 74.68, 73.57, 72.63, 72.50, 71.57, 71.16, 70.64, 70.41, 69.68, 68.16, 62.67, 62.64, 62.57, 61.96, 61.63, 53.11, 23.58, 16.78.

For the purposes of the preparative synthesis of structure 1b a ceramide precursor was attached to the ABC trisaccharide (Scheme 5). Epoxidation of 7b, followed by reaction with the ceramide precursor 17b (as its tributylstannyl ether) promoted by Zn(OTf)₂ provided 20b. Acetylation and PMB removal proceeded smoothly to furnish 21b which is poised for coupling with a suitable DEF trisaccharide donor.

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When trisaccharide 15b was treated with MeOTf in the presence of acceptor 21b, a 4:1 mixture of hexasaccharide isomers was obtained. The major product 2.b was obtained in 50% yield.

5

The ceramide side-chain was elaborated by reduction of the azide functionality using Lindlar's catalyst under an atmosphere H_2 in the presence of palmitic anhydride to provide 18b directly. Desilylation was followed by dissolving metal deprotection of the sulfonamide and benzyl groups and MeOH quench to remove the carbonate and acetate groups. Peracetylation of the crude mixture afforded a 78% yield of peracetylated hexasaccharide. Saponification of this material using NaOMe provided the natural product 1b in 96% yield. The coupling constants and chemical shifts of the anomeric protons of 1b matched reported data. In addition, the product was characterized by exact mass, and 1H and ^{13}C NMR.

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EXAMPLE 5**Synthesis of 20b:**

The benzylated ceramide precursor (475 mg, 1.14 mmol) was dissolved in 4 mL PhH. Bis(tributyltin) ether (0.29 mL, 0.34 g, 0.57 mmol) was added and the reaction vessel (equipped with a Dean-Stark trap) was heated to reflux. After 3 h the reaction was allowed to cool and was concentrated under a flow of N_2 . In a separate flask, the glycal 7b was dissolved in 1 mL anhydrous CH_2Cl_2 and the resulting solution was cooled to $0^\circ C$ and a solution of 3,3-dimethyldioxirane (2.8 mL, 0.25 mmol, 0.09 M in acetone) was added. After 45 min the solution was concentrated under a flow of N_2 , then under vacuum. The tin ether was dissolved in 1 mL anhydrous THF and added via cannula to a mixture of $Zn(OTf)_2$ (170 mg, 0.468 mmol) in 1 mL THF at $-78^\circ C$ (wash 1 x 0.5 mL THF). The reaction was allowed to warm to room temperature over 12 h and

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then was quenched with distilled water. The aqueous phase was extracted 3 x with EtOAc. The combined organic phases were dried over anhydrous MgSO_4 . Flash column chromatography (3:1 hexane/EtOAc, 3 x 16 cm silica gel) afforded 265 mg (66%) of the target compound 20b.

^1H NMR (CDCl_3) δ 7.43-7.15 (m, 45H), 7.03 (d, J = 8.6 Hz, 2H), 6.79 (d, J = 8.6 Hz, 2H), 5.76 (dt, J = 6.7, 15.4 Hz, 1H), 5.43 (dd, J = 8.5, 15.4 Hz, 1H), 5.07 (d, J = 3.5 Hz, 1H), 5.05 (d, J = 12.0 Hz, 1H), 4.90 (d, J = 12.9 Hz, 2H), 4.83-4.77 (m, 3H), 4.69 (d, J = 12.0 Hz, 1H), 4.61 (d, J = 11.9 Hz, 1H), 4.54-4.45 (m, 3H), 4.42-4.25 (m, 7H), 4.18-4.05 (m, 6H), 4.01-3.91 (m, 4H), 3.83 (dd, J = 4.4, 10.6 Hz, 1H), 3.79 (s, 3H), 3.71-3.65 (m, 4H), 3.57-3.32 (m, 7H), 3.20 (m, 1H), 2.29 (bs, 1H), 2.11 (bq, J = 6.7 Hz, 2H), 1.42-1.29 (m, 22H), 0.91 (t, J = 6.6 Hz, 3H); ^{13}C NMR (CDCl_3) δ 158.8, 139.1, 139.0, 138.7, 138.6, 138.34, 138.29, 138.2, 138.1, 130.8, 128.7, 128.55, 128.50, 128.4, 128.33, 128.28, 128.26, 128.12, 128.06, 127.84, 127.76, 127.7, 127.64, 127.60, 127.5, 127.45, 127.36, 125.8, 113.5, 102.7, 100.6, 81.9, 81.5, 79.4, 77.4, 77.0, 76.7, 76.6, 76.4, 75.5, 74.9, 74.7, 74.4, 73.9, 73.3, 73.2, 73.11, 73.06, 72.3, 72.1, 70.0, 69.4, 68.7, 68.1, 67.9, 67.7, 64.2, 55.2, 32.4, 31.9, 29.70, 29.65, 29.5, 29.4, 29.2, 29.0, 22.7, 14.2; IR (thin film) 3447, 3062, 3029, 2923, 2853, 2099, 1612, 1586, 1514, 1496, 1454, 1364 cm^{-1} ; $[\alpha]_D^{23}$ +25.0 (c 0.70).

Synthesis of 21b:

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The above trisaccharide (256 mg, 0.147 mmol) was dissolved in 2 mL anhydrous CH_2Cl_2 . Triethylamine (0.105 mL, 76 mg, 0.753 mmol), DMAP (2 mg, 0.02 mmol) and acetic anhydride (0.042 mL, 45 mg, 0.445 mmol) were added sequentially. The reaction was stirred for 1 h then quenched with saturated aqueous NaHCO_3 . The extracts were dried with anhydrous MgSO_4 , filtered and concentrated.

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Purification by flash column chromatography (4:1 hexane/EtOAc, 2 x 16 cm silica gel) afforded 235 mg (90%) of the desired compound.

5 ^1H NMR (CDCl_3) δ 7.42-7.17 (m, 45H), 7.03 (d, J = 8.6 Hz, 2H), 6.81 (d, J = 8.6 Hz, 2H), 5.75 (dt, J = 6.7, 15.4 Hz, 1H), 5.43 (dd, J = 8.6, 15.4 Hz, 1H), 5.07 (d, J = 3.4, 1H), 4.99-4.90 (m, 4H), 4.85 (d, J = 11.3 Hz, 2H), 4.77 (d, J = 11.9 Hz, 1H), 4.76 (d, J = 12.4 Hz, 1H), 4.70 (d, J = 12.0 Hz, 1H), 4.62 (d, J = 11.7 Hz, 1H), 4.57-4.52 (m, 3H), 4.49-4.34 (m, 7H), 4.30 (d, J = 11.8 Hz, 1H), 4.25 (d, J = 11.8 Hz, 1H), 4.14-4.06 (m, 7H), 4.01-3.95 (m, 2H), 3.91 (dd, J = 5.6, 8.6 Hz, 1H), 3.85 (dd, J = 4.3, 11.1, Hz, 1H), 3.80 (s, 3H), 3.74 (d, J = 9.8 Hz, 1H), 15 3.69 (dd, 7.7, 9.9 Hz, 1H), 3.63-3.51 (m, 5H), 3.43-3.34 (m, 3H), 3.22 (dd, J = 4.6, 8.2 Hz, 1H), 2.12 (dd, J = 6.8, 13.6, 2H), 1.87 (s, 3H), 1.43-1.30 (m, 22H), 0.93, (t, J = 6.6 Hz, 3H); ^{13}C NMR (CDCl_3) δ 169.3, 158.8, 139.1, 139.0, 138.69, 138.65, 138.6, 138.31, 138.26, 138.2, 138.1, 20 138.0, 130.8, 128.8, 128.6, 128.41, 128.35, 128.30, 128.28, 128.14, 128.0, 127.9, 127.8, 127.64, 127.60, 127.58, 127.51, 127.47, 127.38, 126.0, 113.5, 102.7, 100.8, 100.6, 81.5, 79.9, 79.5, 79.4, 79.3, 77.4, 77.1, 76.8, 75.5, 75.3, 74.9, 74.5, 74.2, 73.9, 73.2, 73.1, 25 73.0, 72.4, 72.2, 72.1, 70.2, 69.4, 68.1, 68.0, 67.9, 67.5, 63.8, 55.2, 32.4, 32.0, 29.72, 29.67, 29.5, 29.4, 29.2, 29.1, 22.7, 20.9, 14.2; IR (thin film) 3028, 2923, 2852, 2098, 1751, 1611, 1513, 1496, 1453, 1365, 1232 cm^{-1} ; $[\alpha]_D^{23}$ +20.3 (c 0.45).

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The trisaccharide from above (230 mg, 0.129 mmol) was dissolved in 4 mL CH_2Cl_2 . Distilled water (1 mL) was added and the mixture was cooled to 0°C . DDQ (35 mg, 0.15 mmol) was added and the reaction was stirred for 1 h.

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The reaction was quenched with saturated aqueous NaHCO_3 . The aqueous phase was extracted 3 x with CH_2Cl_2 . The combined organic phases were washed dried over anhydrous

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MgSO₄. Flash column chromatography (4:1 hexane/EtOAc, 2 x 16 cm silica) afforded 182 mg (85%) of the target compound 21b.

5 ¹H NMR (CDCl₃) δ 7.38-7.13 (m, 45H), 5.73 (dt, J= 6.7, 15.4 Hz, 1H), 5.41 (dd, J= 8.6, 15.4 Hz, 1H), 5.09 (d, J= 3.2 Hz, 1H), 4.98 (d, J= 12.5 Hz, 1H), 4.95 (dd, J= 8.0, 9.2 Hz, 1H), 4.87 (d, J= 11.2 Hz, 1H), 4.80 (d, J= 11.3 Hz, 1H), 4.77 (d, J= 10.9 Hz, 1H), 4.70 (d, J= 11.4 Hz, 1H), 4.65-4.50 (m, 6H), 4.45-4.42 (m, 3H), 4.38-4.34 (m, 3H), 4.28 (bs, 2H), 4.15 (d, J= 11.7 Hz, 1H), 4.11 (d, J=11.8 Hz, 1H), 4.08-4.01 (m, 3H), 3.98-3.94 (m, 3H), 3.88 (dd, J= 5.5, 8.5 Hz, 1H), 3.82 (dd, J= 4.3, 7.0 Hz, 1H), 3.77 (dd, J= 3.1, 10.1 Hz, 1H), 3.70 (d, J= 9.8 Hz, 1H), 3.64-3.51 (m, 5H), 3.46 (dd, J= 5.4, 9.4, 1H), 3.39 (m, 1H), 3.34-3.30 (m, 2H), 3.21 (dd, J= 4.7, 8.4 Hz, 1H), 2.09 (m, 2H), 1.90 (s, 3H), 1.84 (d, J= 5.1 Hz, 1H), 1.41-1.27 (m, 22H), 0.90 (t, J= 6.5 Hz, 3H); ¹³C NMR (CDCl₃) δ 169.3, 165.9, 139.3, 138.7, 138.6, 138.5, 138.3, 138.2, 138.1, 138.0, 128.5, 128.4, 128.32, 128.27, 128.25, 128.17, 128.00, 127.94, 127.91, 127.8, 127.75, 127.70, 127.67, 127.61, 127.55, 127.49, 127.45, 127.21, 125.9, 107.8, 102.6, 100.8, 99.4, 81.4, 80.6, 79.3, 77.5, 77.3, 77.0, 76.9, 76.7, 75.5, 75.3, 75.2, 74.3, 73.2, 73.1, 73.0, 72.9, 72.3, 72.1, 70.1, 70.0, 69.1, 68.1, 68.0, 67.8, 67.4, 63.8, 32.4, 31.9, 29.7, 29.6, 29.5, 29.4, 29.2, 29.1, 22.7, 20.9, 14.1; IR (thin film) 3570, 3087, 3062, 3029, 2924, 2853 2099, 1950, 1873, 1752, 1496, 1453, 1366, 1231 cm⁻¹; [α]_D²³ +17.6 (c 1.40).

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EXAMPLE 6

Synthesis of 22b:

35 Thioglycoside 15b (188 mg, 0.151 mmol) and the acceptor 21b (125 mg, 0.0751 mmol) were azeotropically dried with benzene twice. The mixture was then dissolved in 2.6 mL

anhydrous Et₂O and 1.3 mL CH₂Cl₂ and to this solution was added 500 mg of 4 Å mol. sieves. This mixture was stirred for 1 h and then was cooled to 0 °C and MeOTf (0.051 mL, 74 mg, 0.45 mmol) was added. The reaction was stirred at 0 °C for 9 h. Triethylamine (1 mL) was then added and reaction was filtered through a plug of silica and washed with Et₂O. The filtrate was washed with saturated aqueous NaHCO₃ and dried over anhydrous MgSO₄. Purification by preparative HPLC (85:15 hexane/EtOAc) afforded 108 mg (50%) of the target compound 22b. The b/a ratio of the reaction was 4:1.

¹H NMR (CDCl₃) δ 7.75 (d, J= 7.2 Hz, 2H), 7.46-7.05 (m, 63H), 5.75 (dt, J= 6.8, 15.2 Hz, 1H), 5.43 (dd, J= 8.6, 15.5 Hz, 1H), 5.13 (m, 2H), 5.09 (d, 3.6 Hz, 1H), 5.05 (d, J= 11.6 Hz, 1H), 5.00 (d, J= 11.5 Hz, 1H), 4.94-4.86 (m, 5H), 4.83-4.65 (m, 14H), 4.59 (d, 11.7 Hz, 2H), 4.53-4.43 (m, 6H), 4.39-4.31 (m, 4H), 4.23 (d, J= 11.9 Hz, 1H), 4.18 (d, J= 11.9 Hz, 1H), 4.15-4.08 (m, 2H), 4.05-3.57 (m, 31H), 3.54 (d, J= 9.1 Hz, 1H), 3.49-3.45 (m, 2H), 3.38 (m, 1H), 3.31-3.23 (m, 3H), 2.92-2.89 (m, 2H), 2.75 (bt, 6.0 H, H), 2.12 (bq, J= 6.9 Hz, 2H), 1.85 (s, 3H), 1.20-1.09 (m, 42H), 0.92 (t, J= 6.6 Hz, 3H); ¹³C NMR (CDCl₃) δ 169.1, 165.9, 155.5, 140.9, 139.2, 139.0, 138.8, 138.64, 138.59, 138.47, 138.43, 138.3, 138.2, 138.10, 138.07, 138.0, 132.1, 129.1, 128.69, 128.65, 128.56, 128.43, 128.40, 128.36, 128.35, 128.26, 128.17, 128.12, 128.08, 127.97, 127.77, 127.66, 127.64, 127.60, 127.54, 127.49, 127.45, 127.41, 127.3, 126.0, 103.0, 102.7, 100.8, 99.7, 99.2, 98.0, 81.2, 80.6, 79.5, 79.2, 79.0, 78.3, 77.7, 76.8, 76.5, 75.5, 75.3, 75.1, 75.03, 74.97, 74.91, 74.87, 74.0, 73.2, 73.10, 73.07, 72.98, 72.93, 72.6, 72.3, 72.1, 72.0, 71.32, 71.25, 70.2, 69.4, 69.32, 69.25, 68.1, 67.9, 67.5, 68.3, 62.1, 62.0, 56.1, 32.4, 31.9, 29.71, 29.68, 29.66, 29.48, 29.38, 29.2, 29.1, 22.7, 20.7, 18.13, 18.11, 18.01, 17.98, 16.9, 14.2,

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11.9; IR (thin film) 3344, 3030, 2924, 2864, 2101, 1789, 1754, 1496, 1453, 1366, 1232 cm^{-1} .

Synthesis of 18b:

5 The hexasaccharide 22b (66 mg, 0.023 mmol) was dissolved in 1 mL EtOAc. Lindlar's catalyst (66 mg) was added followed by the addition of palmitic anhydride (23 mg, 0.046 mmol). The system was purged under vacuum and then put under 1 atm of H_2 . After 24 h the reaction was
10 filtered through a plug of silica gel, washed with EtOAc, and concentrated. Purification by preparative HPLC (4:1 hexane/EtOAc) afforded 64 mg (90%) of the desired product 18b.

15 ^1H NMR (CDCl_3) δ 7.72 (d, J = 7.2 Hz, 2H), 7.42-7.02 (m, 63H), 5.65 (d, J = 9.1 Hz, 1H), 5.62 (dt, J = 6.6, 15.3 Hz, 1H), 5.31 (dd, J = 8.6, 15.3 Hz, 1H), 5.10 (m, 2H), 5.05 (d, J = 3.6 Hz, 1H), 5.02 (d, J =11.5 Hz, 1H), 4.96 (d, J = 11.4 Hz, 1H), 4.90-4.62 (m, 13H), 4.57-4.38 (m, 8H),
20 4.32-4.26 (m, 3H), 4.21-4.07 (m, 9H), 4.01-3.41 (m, 31H), 3.30 (m, 1H), 3.23 (m, 3H), 2.20 (m, 4H), 1.82 (s, 3H), 1.52 (bm, 2H), 1.32-1.19 (m, 53H), 1.15-1.08 (m, 42H), 0.88 (t, J = 6.8 Hz, 6H); IR (thin film) 3531, 3346, 3063, 3030, 2924, 2854, 1790, 1748, 1674, 1496, 1454, 1365,
25 1236 cm^{-1} ; $[\alpha]^{23}_{\text{D}}$ -17.9 (c 0.65).

EXAMPLE 7**Synthesis of 1b:**

30 The hexasaccharide from above (20 mg, 0.0065 mmol) was dissolved in 0.5 mL THF. A solution of tetrabutylammonium fluoride (1.0 M in THF, 0.050 mL, 0.050 mmol) was added and the reaction was stirred for 2 h. The solution was filtered through a plug of silica, washed
35 with EtOAc and concentrated. The residue was dissolved in 1 mL of anhydrous MeOH and NaOMe (10 mg, 0.19 mmol) was added. The reaction was stirred for 3 h, neutralized

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with 40 mg of Dowex-50 resin, filtered and concentrated. Purification by flash column chromatography (1.5 x 4 cm 10-40 u silica gel, 95:5 CH₂Cl₂/MeOH) afforded 16.5 mg (94%) of the desired compound.

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¹H NMR (CDCl₃) δ 7.78 (d, J= 7.6 Hz, 2H), 7.46 (d, J= 7.4 Hz, 2H), 7.41-6.97 (m, 61H), 6.02 (d, J= 9.1 Hz, 1H), 5.76 (bs, 1H), 5.67 (dt, J= 6.6, 15.3 Hz, 1H), 5.37-5.30 (m, 2H), 5.19 (d, J= 2.6 Hz, 1H), 4.96 (d, J= 11.3 Hz, 1H), 4.93 (d, J= 3.4 Hz, 1H), 4.90-4.83 (m, 3H), 4.78-4.66 (m, 7H), 4.56 (d, J= 11.1 Hz, 1H), 4.53 (d, J= 10.2 Hz, 1H), 4.47-4.32 (m, 5H), 4.28-4.06 (m, 14H), 4.01-3.13 (m, 36H), 2.73 (bt, 1H), 2.61 (bs, 1H), 2.54 (bs, 1H), (2.05 (m, 4H), 1.50 (m, 2H), 1.38-1.23 (m, 46H), 0.88 (t, J= 6.6 Hz, 6H), 0.78 (d, 6.3 Hz, 3H); ¹³C NMR (CDCl₃) δ 173.4, 142.4, 139.5, 139.0, 138.7, 138.5, 138.33, 138.26, 138.14, 138.09, 137.9, 137.2, 137.1, 131.6, 129.0, 128.8, 128.54, 128.47, 128.37, 128.32, 128.27, 128.22, 128.17, 128.14, 128.05, 127.99, 127.79, 127.73, 127.68, 127.63, 127.59, 127.49, 127.46, 127.37, 127.32, 126.98, 126.58, 104.1, 102.83, 102.76, 100.3, 100.2, 82.1, 81.5, 81.2, 79.6, 79.2, 79.0, 78.0, 77.3, 77.0, 76.7, 75.6, 75.3, 75.1, 75.0, 74.8, 74.6, 73.5, 73.4, 73.2, 73.0, 72.7, 72.6, 71.9, 70.1, 69.6, 68.5, 68.2, 68.0, 67.5, 62.4, 61.9, 54.8, 52.3, 36.9, 32.3, 31.9, 29.71, 29.67, 29.54, 29.50, 29.43, 29.37, 29.28, 29.20, 25.7, 22.7, 16.7, 14.1; IR (thin film) 3424, 3062, 3023, 2923, 2852, 1641, 1530, 1496, 1453, 1362, 1325 cm⁻¹; [α]_D²³ -3.2 (c 0.83).

30

A flask was equipped with a dry ice condenser and was charged with 4 mL NH₃. Sodium (18 mg, 0.78 mol) was added and to the resulting blue solution was added 29 mg of the above hexasaccharide (0.010 mmol). The reaction was stirred at -78°C for 45 min. Quench by the addition of MeOH (3 mL). Nitrogen was blown over the solution to evaporate the NH₃. The reaction was neutralized with 170 mg of D wex-50 resin, filtered and concentrated. The

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resulting residue was dissolved in 1 mL of 4:1 THF/DMF. Triethylamine (0.5 mL) was added followed by the addition of DMAP (3 mg) and acetic anhydride (0.200 mL). After 2 h the reaction was concentrated in vacuo. Purification by flash column (1.5 x 5 cm 10-40 m silica, 9:1 EtOAc/hexane) afforded 18 mg (78%) of the peracetate. A sample of this hexasaccharide (15 mg, 0.0065 mmol) was dissolved in 0.5 mL of anhydrous MeOH and a NaOMe solution (30% in MeOH, 0.010 mL, 0.05 mmol) was added. The solution was stirred for 3 h, neutralized with 9 mg Dowex-50 resin, filtered and concentrated. The residue was purified by flash column chromatography (1.5 x 4 cm C-18 reverse phase silica, MeOH) to afford 9.6 mg of the natural product 1. Spectral data agree with those reported by Hakomori, et al.

EXAMPLE 8

Synthesis of 3a and 6a

3a: To 2.00g (2.47 mmol) of lactal carbonate 2a was added 4.44g (9.86 mmol) of fucosyl fluoride 5a. The mixture was azeotroped 5 times with benzene and placed under high vacuum for two hours. Under an argon atmosphere 2.77 ml (12.33 mmol) of di-tert-butyl pyridine and 16ml of dry ether were added. 2.0 g of freshly activated 4A molecular sieves were added and the mixture stirred one hour at room temperature. In an argon glove bag, 2.34g (12.33 mmol) of stannous chloride (SnCl_2) and 2.56g (12.33 mmol) of silver perchlorate (AgClO_4) were added. The flask was equipped with a reflux condensor and the reaction brought to reflux for 72 hours. The reaction was quenched with 5ml of saturated bicarbonate and filtered through a pad of celite. Diluted with 50ml ethyl acetate and washed 2 times with sat. bicarbonate, 2 times with sat. copper sulfate and 2 times with sat. brine. The organics were dried over MgSO_4 and concentrated. Flash chromatography in 20% ethyl acetate/hexanes afforded 2.10g (51%) of a

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white f am 3a: $[\alpha]_D -78.9$ (c.555, CHCl₃); IR (thin film) 3040, 3000, 2905, 2860, 2830, 1820, 1800, 1710, 1635, 1585, 1570, 1480, 1460, 1440, 1415, 1370, 1350, 1300, 1260, 1205, 1145, 1100, 950, 735, 695, ¹ H NMR (400MHz, CDCl₃) δ 8.09 (d, $J = 8.12$ Hz, 2H), 8.00 (d, $J = 8.26$ Hz, 2H) 7.66 (m, 4H), 7.59 (d, $J = 6.74$ Hz, 4H), 7.56 (t, $J = 7.27$ Hz, 1H), 7.30-7.50 (m, 22H) 7.16-7.26 (m, 10H) 7.09 (m, 2H), 6.99 (t, $J = 7.59$ Hz, 2H) 6.89 (t, $J = 7.97$ Hz, 1H), 6.43 (d, $J = 6.08$ Hz, 1H), 5.46 (bs, 1H), 5.38 (bs, 1H), 5.35 (d, $J = 3.42$ Hz, 1H), 4.89 (d, $J = 11.35$ Hz, 1H), 4.75-4.80 (m, 4H), 4.72 (d, $J = 5.88$ Hz, 2H), 4.69 (d, $J = 4.27$ Hz, 2H), 4.36-4.55 (m, 5H), 4.28 (q, $J = 6.51$ Hz, 1H), 4.17 (bd, $J = 5.46$ Hz, 1H), 3.90-4.00 (m, 6H), 3.85 (d, $J = 2.99$ Hz, 1H), 3.82 (d, $J = 2.89$ Hz, 1H), 3.56-3.78 (m, 4H), 1.07 (m, 24H); HRMS (FAB): calcd for C₉₉H₁₀₆O₂₀Si₂Na 1694.6740 found 1694.6787.

6a: 230 mg (0.12mmol) of iodosulfonamide 4a was azeotroped 5 times with dry benzene and placed under high vacuum for two hours. Added 2.4ml of THF solution of 15eq. of tin ether 9a (generated by azeotropic removal of water overnight with a Dean-Stark trap equipped with freshly activated 4A mol. sieves from 561 mg (1.80mmol) of 6a-TIPS-galactal and 673 μ l (1.32mmol) bis(tributyltin) oxide in 80 ml of benzene). To this solution stirring under an argon atmosphere was added 200 mg of freshly activated 4A powdered molecular sieves. Stirred one hour at room temperature. Cooled solution to -78°C and added, via cannula, a solution of 187 mg (.96mmol) of silver tetrafluoroborate in 2.4 ml of THF. Warmed to room temperature over 15 hours and quenched the reaction, which had turned bright yellow, with 2ml. of sat. bicarbonate. The reaction mixture was filtered through a pad of celite into a separatory funnel. The celite pad was washed thoroughly with ethyl acetate. The organics were washed twice with sat. bicarbonate and twice with sat. brine. The organics were dried over MgSO₄.

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Concentration and chromatography in 25% ethyl acetate/hexanes gave 193 mg (75%) as a white foam 6a: $[\alpha]_D -126.4^\circ$ (c, 505, CHCl_3), IR (thin film) 3500, 3040, 3000, 2905, 2840, 1820, 1800, 1705, 1635, 1590, 1440, 1410, 1255, 1195, 1100, 1080, 1035, 815, 730, 695; ^1H NMR (400 MHz, CDCl_3) δ 8.09 (app t, 4H), 7.08-7.65 (m, 46H), 6.90 (t, $J=7.65$ Hz, 3H), 6.76 (d, $J=6.91$ Hz, 2H), 6.12 (d, $J=6.59$ Hz, 1H), 5.50 (bs 1H), 5.45 (bs 1H), 5.28 (app t, 2H), 3.03-4.91 (m, 36H), 1.09 (m, 45H); LRMS (FAB): calcd for $\text{C}_{120}\text{H}_{141}\text{NO}_{26}\text{SSi}_3\text{Na}$ 2153 found 2153.

EXAMPLE 9

- 6: ^1H NMR (400 MHz, CDCl_3); δ 6.39 (d, 1H, $J=6.2$ Hz, H_1 galactal), 5.65 (d, 1H, $J=8.9$ Hz, NHAc), 5.35 (d, 1H, $J=3.8$ Hz), 5.33 (m, 1H), 5.29 (d, 1H, $J=2.6$ Hz), 5.27 (d, 1H, $J=3.1$ Hz), 5.17-5.09 (m, 2H), 4.97-4.90 (m, 2H), 4.81 (dd, 1H, $J=3$ Hz, $J=6.1$ Hz, H_2 galactal), 4.75 (d, 1H, $J=8.0$ Hz), 4.52 (m, 1H), 4.48 (dd, 1H, $J=12.0$ Hz), 4.44-4.06 (m, 8H), 3.88-3.77 (m, 4H), 3.61 (m, 1H), 2.18-1.97 (m, 33 H, COCH_3), 1.18 (d, 3H, $J=6.5$ Hz, CH_3 fucose); ^{13}C NMR (CDCl_3): δ 170.80, 170.77, 170.72, 170.67, 170.62, 170.34, 170.21, 170.09, 170.01, 169.99, 169.65, 144.92 (C_1 galactal), 100.22, 98.83, 98.58, 95.55, 74.48, 73.38, 73.13, 73.06, 71.48, 71.01, 70.68, 67.97, 67.42, 67.18, 67.05, 65.94, 64.83, 62.35, 62.22, 60.88, 60.37, 54.21, 23.23, 22.15, 20.85, 20.82, 20.79, 20.76, 20.65, 20.61, 20.57, 15.51, (C_6 fucose); IR (thin film): 3368.7 (NH), 2965.6, 2934.6, 1746.5 (C=O), 1537.5, 1435.9, 1371.3, 1228.5, 1065.0, 1046.0; $[\alpha]_D^{25} = -51.1^\circ$ (c 1.8, CH_2Cl_2); HRMS (FAB); calcd. for $\text{C}_{46}\text{H}_{63}\text{NNaO}_{28}$: $m/z = 1100.3434$, found 1100.3436.
- 21: Polymer-bound galactal 7 (loading = 0.85 mmol glycal/g), which had been placed in a round-bottom flask equipped with a fritted outlet, was suspended in CH_2Cl_2

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under N₂, cooled to 0°C, and then treated with a solution of 3,3-dimethyldioxirane. The mixture was stirred (teflon-coated magnetic stir bar) for 40 min. at 0°C, after which time solubles were removed by filtration through the fritted outlet (N₂ pressure). The polymer bound 1,2 anhydrosugar was evacuated (ca. 0.1 torr) for several hours in order to dry the material for the next step. This material was once again placed under N₂ before being treated with 19 (~10 molar equivalents as a 0.5 M solution in THF). The suspension was cooled to -40 °C, and treated with ZnCl₂ (~2 molar equivalents as a 1.0 M solution in THF). The reaction mixture was allowed to slowly warm to rt (over ca. 2 h), and then stirred an additional 3-4 h. Solubles were removed by filtration, and polymer 18 was washed several times with THF and then dried in vacuo. To compound 18 was added, in a glove bag, solid Sn(OTf)₂ (~ molar equivalents), and the mixture was placed under N₂ and cooled to 0°C before being treated with 10 (~5 molar equivalents as a 0.2 M solution in THF and di-tert-butylpyridine (~8 molar equivalents). The suspension was allowed to warm to rt and stirred 8-10 h. The mixture was rinsed with anhydrous THF (2 times), 1,4-dioxane (2 times), again with THF, and then dried in vacuo. Compound 20 (100 mg) was suspended in THF, treated with a 1:3 mixture of AcOH and TBAF (~0.2 M in TBAF, ~10 molar equivalents), and the mixture was stirred for 18 h at 40 °C. The polymer was rinsed with THF (3 times), and the combined rinsings were concentrated and purified by column chromatography on silica gel (1:1 EtOAc: hexanes). Compound 21 (18 mg) was obtained as a colorless solid (40% overall yield from 7): ¹H NMR (400 MHz, CDCl₃): δ 7.40-7.25 (m, 30H, Ar H), 6.18 (d, 1H, J= 6.0 Hz, H₁ glucal), 5.26 (d, 1H, J=3.5 Hz, H₁ fucose), 5.09 (d, 1 H, J= 3.7 Hz, H₁ fucose), 4.96 (t, 2 H, J= 10.8 Hz, PhCH₂), (4.90-4.56 (m, 13 H), 4.43 (m, 1H), 4.15-4.06 (m, 4 H), 3.97 (dt, 1 H, J=8.3 Hz, J=2.4 Hz), 3.87-3.65 (m, 10H), 3.64 (d, 1 H), 3.57 (d, 1 H), 2.69 (br, 1 H,

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OH), 2.52 (br, 1 H, OH), 1.11 (d, 3 H, $J = 7.0$ Hz, CH₃ fucose), 1.09 (d, 3H, $J = 7.0$ Hz, CH₃ fucose); ¹³C NMR (CDCl₃); δ 153.37 (C=O), 145.75 (C₁ glucal), 138.60, 138.52, 138.19, 137.61, 128.55, 128.52, 128.44, 128.24, 128.16, 128.07, 127.62, 127.56, 127.45, 98.71, 98.38, 97.65, 97.34, 79.26, 78.87, 78.67, 78.01, 77.79, 77.65, 76.37, 76.10, 74.92, 74.40, 74.16, 73.95, 72.86, 72.64, 72.53, 67.43, 67.29, 61.31, 60.90, 16.65 (C₆ fucose), 16.53 (C₆ fucose); IR (thin film): 3467.0 (OH), 3029.6, 2923.6, 1807.2 (C=O), 1647.3, 1496.0, 1453.5, 1358.1, 1240.2, 1095.6, 1049.2, 738.5, 697.2; $[\alpha]_{D23} = -82.5^\circ$ (c 0.4, CH₂Cl₂); HRMS (FAB); calcd. for C₆₇H₇₄NaO₁₈: $m/z = 1189.4772$, found 1189.4757.

25: To a mixture of 23 (60 mg, 34 μ mol) and powdered 4A molecular sieves (200 mg) under N₂ was added, via canula, a solution of 24 (0.21 mmol) in anhydrous THF (1.5 mL). The stirred suspension was cooled to -78°C before being treated with a solution of AgBF₄ (0.21 mmol) in 0.25 mL of anhydrous THF. The mixture was stirred and allowed to slowly warm to rt overnight. The suspension, which had developed a bright-yellow color, was heated, with stirring, at 45°C for an additional 36 h, until the TLC (2.5 EtOAc:hexanes) showed no trace of 23. The mixture was treated with saturated aqueous NH₄Cl (5 mL) and then extracted with EtOAc (3 x 10 mL), and the organics were dried over MgSO₄. The crude product was purified by silica gel chromatography (1:3 EtOAc:hexanes) to give 25 as a colorless oil (42 mg, 55%): ¹H NMR (400 MHz, acetone-d₆): δ 8.17(d, 2 H, $J = 7.3$ Hz, PhSO₂), 7.50-7.20 (m, 33H, ArH), 6.52 (d, 1 H, $J = 10.5$ Hz, NH), 6.30 (dd, 1 H, $J = 6.0$ Hz, H₁ glucal), 5.35-5.32 (m, 2H), 5.25 (d, 1H, $J = 7.9$ Hz), 5.15 (m, 2 H), 4.99-4.92 (m, 3H), 4.86-4.52 (m, 14 H), 4.45 (dd, 1H, $J = 7.91$ Hz, $J = 2.4$ Hz), 4.32-4.23 (m, 3H), 4.22 (dd, 1 H), 4.17 (d, 1 H, $J = 10.1$ Hz), 4.08-3.84 (m, 18 H), 3.79-3.73 (m, 2H), 3.66 (m, 1H), 3.55 (t, 1 H, $J = 6$ Hz), 3.50 (dd, 1 H, $J = 9.7$ Hz), 1.33 (d, 3 H, $J = 6.5$ Hz, CH₃ fucose), 1.31 (d, 3H, $J = 6.4$ Hz, CH₃

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- fucose), 1.20-0.98 (m, 84 H, 3 x Si(i-Pr)₃); ¹³C NMR (acetone-d₆): 145.66 (C=O), 132.72, 131.48, 131.45, 131.28, 131.16, 130.77, 130.48, 121.31, 120.11, 119.86, 119.78, 119.25, 95.63, 94.70, 91.37, 89.64, 89.31, 86.52, 73.38, 72.24, 71.00, 70.71, 70.37, 69.80, 69.59, 69.06, 68.23, 67.92, 67.38, 67.10, 66.49, 65.67, 65.33, 64.60, 64.34, 64.03, 63.45, 63.30, 59.46, 58.83, 58.37, 54.45, 53.32, 49.86, 19.67 (C₆ fucose), 18.42 (C₆ fucose), 9.55, 9.48, 9.45, 9.31, 9.23, 3.82, 3.70, 3.64; IR (thin film): 3491.9 (OH), 3030.1, 2941.2, 2865.5, 1835.8, 1819.5, 1649.8, 1496.2, 1462.3, 1349.9, 1245.5, 1155.2, 1095.1, 1049.4, 882.2, 734.8, 692.0; [α]_{D23} = -33.8° (c 2.0, CH₂Cl₂); HRMS (FAB): calcd for ¹²C₁₂₀¹³CH₁₇₉NNaO₂₉SSi₄: m/z = 2278.1292, found 2278.1296.
- 17: ¹H NMR (400 MHz, CD₃OD): δ 6.00 (m, 1H, J = 5.6 Hz, CH₂CH=CH₂), 5.37 (dd, 1 H, J = 1.6 Hz, J = 7.3 Hz, CH₂CH=CH₂), 5.20 (dd, 1 H, J = 1.6 Hz, J = 9.5 Hz, CH₂CH=CH₂), 5.18 (d, 1 H, J = 3.9 Hz, H₁ fucose), 5.10 (d, 1H, J = 3.8 Hz, H₁ fucose), 4.64 (d, 1 H, J = 6.9 Hz), 4.45 (d, 1H, J = 7.4 Hz), 4.43-4.23 (m, 2H), 4.27 (dd, 1H, J = 9.3 Hz, J = 10.6 Hz), 4.23-4.11 (m, 2H), 4.02-3.29 (m, 31 H), 2.06 (s, 3H, NAc), 1.31 (d, 3H, J = 6.6 Hz, CH₃ fucose), 1.29 (d, 3 H, J = 6.6 Hz, CH₃ fucose); ¹³C NMR (CD₃OD): δ 173.20 (C=O), 135.73 (CH₂CH=CH₂), 105.13, 103.30, 102.49, 101.62, 99.63, 96.86, 80.79, 80.67, 78.44, 76.67, 76.49, 75.89, 74.80, 74.59, 73.94, 73.61, 73.40, 71.55, 71.38, 71.16, 70.42, 70.26, 70.14, 67.77, 67.30, 67.21, 62.79, 62.34, 61.99, 55.54, 22.97 (NAc), 16.65 (2 C's, C₆ fucose); IR (thin film): 3376.6 (OH), 2924.2, 1652.5 (C=O), 1383.1, 1032.4; [α]_{D23} = -12.8° (c 0.25, MeOH); HRMS (FAB): calcd. for C₄₁H₆₉NNaO₂₉: m/z = 1062.3853, found 1062.3837

35 Results and Discussion

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A Highly Convergent Synthesis of the Lewis X Blood Group
Determinant in Conjugatable Form

Construction of the Le^x determinant commences with lactal
5 (1a) (W.N. Haworth, E.L. Hirst, M.M.T. Plant, R.J.W.
Reynolds, *J. Chem. Soc.* 1930, 2644) as shown in Figure 2.
Capping both primary hydroxyl groups as their TBDPS
ethers under standard conditions was followed by simple
engagement of the 3' and 4' hydroxyl functions as a
10 cyclic carbonate 2a. The stereospecific introduction of
two α -linked fucose residues gave tetrasaccharide glycal
3a in 51% yield in a single step. The donor used was the
known fluorosugar 5a (S.J. Danishefsky, J. Gervay, J.M.
Peterson, F.E. McDonald, K. Koseki, T. Oriyama, D.A.
15 Griffith, C-H. Wong, D.P. Dumas, *J. Am. Chem. Soc.* 1992,
114, 8329) following a modification of the original
Mukaiyama conditions. (T. Mukaiyama, Y. Murai, S. Shoda,
Chem. Lett. 1981, 431) Glycal 3a corresponds to the Le^x
hapten, lacking the N-acetyl function in the glucose
20 residue. The problem was then to introduce this group as
well as a galactose spacer module.

Methodology developed previously (D.A. Griffith, S.J.
Danishefsky, "On the Sulfonamidoglycosylation of Glycals.
25 A Route to Oligosaccharides With 2-Aminohexose
Subunits+", *J. Am. Chem. Soc.* 1990 112, 5811) proved
appropriate to attain these goals. Glycal 3a was treated
with iodonium dicollidine perchlorate and
benzenesulfonamide to afford iodosulfonamide 4a.
30 Azaglycosylation using the 3-stannyl ether of galactal
(9a) (S.J. Danishefsky, K. Koseki, D.A. Griffith, J.
Gervay, J. M. Peterson, F.E. McDonald, T. Oriyama, *J. Am.
Chem. Soc.* 1992, 114, 8331) in the presence of silver
tetrafluoroborate gave pentasaccharide glycal 6a in 75%
35 yield as shown in Figure 3. Having 6a in hand, one can
iterate the azaglycosylation sequence or activate the
glycal as its epoxide and continue with further

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glycosylations. To demonstrate the ability to fashion a conjugatable form of Le^y hapten, formation of the allyl glycoside was important. The feasibility of converting the sulfonamido group into the target acetamide was demonstrated. Glycal 6a was deprotected in two steps as shown. Peracetylation afforded acetamido glycal 7a. Activation of the glycal as its epoxide with dimethyldioxirane (R.L. Halcomb, S.J. Danishefsky, *J. Am. Chem. Soc.* 1989, 111, 6661), followed by epoxide opening with allyl alcohol in the presence of zinc chloride gave the desired peracetylated β -allyl pentasaccharide which was deacetylated by action of methoxide to provide the target Le^y hapten as its β -allyl glycoside 8a. (8a $[\alpha]_D -72.7^\circ$ (c. 1 MeOH); IR (thin film) 3350, 2940, 2900, 2830, 1650, 1550, 1365, 1300, 1155, 1070, 1030; ¹H NMR (400 MHz, CD₃OD) δ 5.95 (m, 1H), 5.32 (d, $J=17.25$ Hz, 1H), 5.14-5.19 (m, 2H), 5.04 (d, $J=3.83$ Hz, 1H), 5.02 (d, $J=3.50$ Hz, 1H), 4.68 (d, $J=8.15$ Hz, 2H), 4.51 (d, $J=5.70$ Hz, 1H), 3.40-4.38 (m, 27H), 1.96 (s, 3H), 1.23 (m, 6H); HRMS (FAB) calcd for C₃₅H₅₆NO₂₄Na 900.3325 found 900.3310) The aldehyde, derived by ozonolysis of 8a, could be conjugated to a carrier protein by the method of Bernstein and Hall.

This synthesis is the most direct route to the Le^y determinant known. (O. Hindsgaul, T. Norberg, J. Le Pendu, R. U. Lemieux, *Carbohydr Res.* 1982, 109, 109; U. Spohr, R.U. Lemieux *ibid*, 1988, 174, 211; for previous syntheses, see: J.C. Jacquinet, P. Sinay, *J. Org. Chem.* 1977, 42, 720; S. Nilsson, H. Lohn, T. Norberg, *Glycoconjugate J.* 1989, 6, 21; R.R. Schmidt, A. Topfer, *Tetrahedron Lett.* 1991, 32, 3353; W. Kinzy, A. Low, *Carbohydrate. Res.* 1993, 245, 193) The method is stereospecific at each step, and it illustrates the versatility of glycals both as donors and acceptors and takes advantage of 1,2-glycal epoxides and their presumed N-sulfonylaziridine counterparts. The method also makes

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possibl extensive analogue preparation and variation of conjugation strategies.

5 **A Strategy for the Assembly of Complex, Branched Oligosaccharide Domains on a Solid Support: An Application to a Concise Synthesis of the Lewis^b Domain in Bioconjugatable Form.**

10 The assembly of the Le^b (type 1) domain is a relatively more difficult undertaking than was the Le^y (type 2) target, wherein lactal was used as a convenient starting material. In the case of the type 1 determinant, lactal is not a useful starting material. The synthesis of the Le^b system offered an opportunity to apply the polymer-
15 based oligosaccharide construction method. (S.J. Danishefsky, K.F. McC lure, J.T. Randolph, R.B. Ruggeri, *Science* 1993, 260, 1307) The strategy is summarized in Figure 4, wherein polymer-bound glycal 1 is activated for glycosyl donation via direct formation of a 1,2-anhydro
20 derivative 2. Reaction of 2 with acceptor glycal 3 furnishes 4. Reiteration is achieved by means of direct epoxidation and reaction with acceptor 3. The self-policing nature of the method and the simple "one time" purification at the end of the synthesis are useful
25 features.

30 The present invention discloses an important additional dimension of the polymer-bound method. The logic is captured by inspection of Figure 5. Each glycosylation event generates a unique C₂ hydroxyl. In principle (and in fact, see *infra*) this hydroxyl can function as a glycosyl acceptor upon reaction with a solution based donor. The glycal linkage of 5, still housed on the support, can be further elongated. In this way,
35 branching at C₂ is accomplished while minimizing the requirement for protecting group machinations. (For an application of this strategy in the synthesis of a

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complex saponin, see: J.T. Randolph, S.J. Danishefsky, *J. Am Chem Soc.* 1993, 115, 8473)

5 In principle, this branching can be implemented at any site in a growing chain. For such an extension, it would be necessary to cap all previously generated hydroxyl groups generated on the "polymer side" (non-reducing end) of the growing domain. Thus, the polymer-bound oligosaccharide can serve as either donor or acceptor, 10 wherever appropriate.

Initial efforts at reduction to practice identified tetrasaccharide glycal 6, bearing H-type 2 blood group specificity, as a goal. Polymer-supported galactal 7 15 (using as polymer support polystyrene crosslinked with 1% divinylbenzene functionalized using published procedures: T-H. Chan, W.-Q. Huang, *J. Chem. Soc., Chem. Commun.* 1985, 909; M.J. Farrall, J.M.J. Frechet, *J. Org. Chem.* 1976, 41, 3877) reacted with a solution of 3,3-dimethyldioxirane (R.W. Murray, R. Jeyaraman, *J. Org. Chem.* 1985, 50, 2847), to provide the corresponding 1,2-anhydrosugar glycosyl donor, which was treated with a solution of glucal derivative 8 in the presence of ZnCl_2 to provide 9 (R.L. Halcomb, S.J. Danishefsky, *J. Am. Chem Soc.* 1989, 111, 6661) This polymer-bound disaccharide 25 acted as a glycosyl acceptor upon treatment with a solution of fucosyl fluoride 10 (K.C. Nicolau, C.W. Hummel, Y. Iwabuchi, *J. Am. Chem. Soc.* 1992, 114, 3126) in the presence of $\text{Sn}(\text{OTf})_2$ thereby giving 11. Retrieval 30 of the trisaccharide glycal from the support was accomplished using tetrabutylammonium fluoride (TBAF) to afford 12 in 50% overall yield from 7.

35 The trisaccharide, retrieved from the polymer, could then be further elaborated. Toward this end, compound 12 was converted to silyl ether 13 by reaction with TIPSCl. The latter was converted to the iodosulfonamide derivative 14

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- by the action of $I(coll)_2ClO_4$ in the presence of $PhSO_2NH_2$. Reaction of 14 with galactal stannyl ether derivative 15 in the presence of $AgBF_4$ gave 16 77% yield. (D.A. Griffith, S.J. Danishefsky, *J. Am. Chem Soc.* 1990, 112, 5811) Tetrasaccharide glycal 16 was deprotected and peracetylated to afford 6. (S.J. Danishefsky, K. Koseki, D.A. Griffith, J. Gervay, J.M. Peterson, F.E. McDonald, T. Oriyama, *J. Am. Chem Soc.* 1992, 114, 8331)
- 10 Thus, the synthesis of the full H-type determinant was achieved by sequential polymer- and solution-based maneuvers. The next target was the more complex Le^b hexasaccharide 17. The campaign proceeded as shown in Figure 6. Polymer-bound galactal 7 was converted to 18
15 upon epoxidation with 3,3-dimethyldioxirane followed by reaction with glucal derivative 19. This disaccharide diol was then bisfucosylated using fucosyl donor 10 in the presence of $Sn(OTf)_2$ to afford 20. Retrieval from the support with TBAF provided 21, which was obtained in 40%
20 overall yield from 7. Compound 21 reacted with TIPSCl to give 22.
- Iodosulfonamide 23, obtained from 22 using $I(coll)_2ClO_4$ and $PhSO_2NH_2$, reacted with lactal derivative 24 in the
25 presence of $AgBF_4$ to provide hexasaccharide glycal 25 in 55% yield. Deprotection of 25 was accomplished in two stages (TBAF to remove the silyl ethers, followed by Na/NH_3 reduction to remove the aromatic protecting groups), and the crude product was peracetylated to give
30 26 in a 51% overall yield. Compound 26 was converted, via the 1,2-anhydrosugar derivative, to allyl glycoside 17, which can be activated by ozonolysis to the aldehyde ($R = CH_2CHO$) for subsequent coupling to a protein by the method of Bernstein and Hall.
- 35 In sum, the present invention extends the solid-support glycal assembly method for complex carbohydrate domain

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synthesis to include the branching patterns critical for biorecognition. Specifically, the determinant for the binding of *H. pylori* to human gastric epithelium has been stereospecifically fashioned in a way which reduces need
5 for protecting groups.

Glycal Assembly Method Applied to the Synthesis of Human Breast Tumor-Associated Antigen

10

The present invention provides a convergent synthesis of the hexasaccharide wherein the two trisaccharide domains have been efficiently assembled in forms readily amenable for coupling. The synthesis of the ABC
15 trisaccharide is presented in Figure 8. The α -linkage of this trisaccharide might be formed by employing a fluoro-sugar donor 4b, using established conditions. (Gordon, D. M.; Danishefsky, S. J., *Carbohydr. Res.*, 1990, 206, 361-366.) Preparation of the appropriate disaccharide
20 acceptor commenced with 5b (Danishefsky, S. J.; Behar, V.; Randolph, J. T.; Lloyd, K. O., *J. Am. Chem. Soc.*, 1995, 0000), itself obtained from a glycal coupling. Benzylation followed by desilylation, carbonate removal and selective dibenzylation afforded the disaccharide
25 6b. The acceptor thus obtained was reacted with the fluorosugar 4b using modified Mukaiyama conditions (Mukaiyama, T.; Murai, Y.; Shoda, S., *Chem. Lett.*, 1981, 431-433) to provide the trisaccharide glycal 7b. Deprotection of the PMB ether provided the ABC
30 trisaccharide 8b, which was poised for coupling with a suitable DEF trisaccharide donor.

The synthesis of the DEF trisaccharide is described in Figure 9. Epoxidation of the galactal 9b and standard
35 coupling (Halcomb, R.L.; Danishefsky, S.J., *J. Am. Chem. Soc.*, 1989, 111, 6661-6666.) with acceptor 10b afforded, regioselectively, the disaccharide 11b. Fucosylation

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employing the fluoro-fucose 12b (Dejter-Juszynski, M.; Flowers, H.M., *Carbohydr. Res.*, 1973, 28, 61) provided a 5:1 ratio of monoglycosylation regioisomers, the major isomer being the desired trisaccharide 13b. This material was treated under standard conditions to afford the trans-diaxial iodosulfonamide 14b.

Direct coupling reactions (Griffith, D.A.; Danshefsky, S.J., *J. Am. Chem. Soc.*, 1990, 112, 5811-5819; Danishefsky, S.J.; Koseki, K.; Griffith, D.A.; Gervay, J.; Peterson, J.M.; McDonald, F.E.; Oriyama, T., *J. Am. Chem. Soc.*, 1992, 114, 8331-8333) employing iodosulfonamides such as 14b with ABC trisaccharide acceptors failed, leading to a different donor functionality in the trisaccharide. In practice, the iodosulfonamide 14b was treated with excess lithium ethanethiolate to afford the ethyl thioglycoside 15b (Figure 10). Precedent established by the present inventors lead to the prediction of sulfonamide participation to provide the desired β -linked product from 15b. (Griffith, D.A., Ph.D. Thesis, Yale University, 1992) When donor 15b was treated with MeOTf in the presence of acceptor 8b, a 10:1 mixture of hexasaccharide isomers was obtained. The major product 16b was obtained in 70-85% yield.

Ceramide attachment and elaboration commenced with epoxidation of 16b, followed by reaction with the stannyl ether 17b promoted by $\text{Zn}(\text{OTf})_2$. (Liu, K.K.-C.; Danishefsky, S.J., *J. Am. Chem. Soc.*, 1993, 115, 4933-4934) Although the yield of this ceramide coupling is low, when this reaction was performed on trisaccharide 7b, the corresponding product was obtained in 66% yield. This material can then be used to obtain 18b. Following acetylation, the ceramide side-chain was elaborated by reduction of the azide functionality using Lindlar's catalyst under an atmosphere of H_2 in the presence of

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palmitic anhydrid to provide 18b. Desilylation and saponification was followed by dissolving metal deprotection and MeOH quench. Peracetylation of the crude mixture, followed by saponification provided the glycosphingolipid 1b. Only the chemical shifts and coupling constants of the anomeric protons have been reported for the natural material. The spectrum of synthetic 1b is in complete agreement with this data. Furthermore, the product was characterized by exact mass, and ^1H and ^{13}C NMR. The synthetic material has also been shown to bind to the monoclonal antibody MB1.

In addition, the present invention provides the corresponding allyl glycoside (Figure 11). Deprotection of 16b, as above, and acetylation afforded the peracetate of the hexasaccharide glycal. Epoxidation, reaction with allyl alcohol, and saponification provided the allyl glycoside 19b.

As in the case of the Le determinant, ozonolysis of the allyl group of 19b prepares the compound for reductive coupling to lysine residues of proteins.

Biological Results

The MB1 hexasaccharide has been prepared in two forms, the natural "B" form and the unnatural "A" form as shown below.

The natural structure ("β") is:

Fucal-GalB1-3GalNACB1-3Galα1-4G1B1-4GcB1-1Cer

The unnatural structure "α" is:

Fucα1-2GalB1-3GalNACα1-3Galα1-4GalB1-1Cer

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Both have been linked to ceramide to facilitate testing for immunological reactivity with monoclonal antibody (mAb) MB1.

5 By Thin Layer Chromatography (TLC) the 2 preparations migrate as similar single bands. Immune TLC (see Ritter, G., et al., *Cancer Res.*, 1990, 50, 1403-10)) demonstrates that both forms react with the MB1 monoclonal antibody specifically but that the β -form reacts 10 times more
10 strongly (comparable staining is seen with 1/10 the amount of antigen). The high level of reactivity of the β structure with mAb MB1 was confirmed using flow cytometry inhibition assays. Reactivity of MAb MB1 with breast cancer cell lines such as MCF-7 was 98% inhibited
15 by 8 μ g/ml of the β linkage preparation but was only 6% inhibited by 8 μ g of the α -linkage preparation. GD3 ganglioside (negative control) showed no inhibition at all.

20 **Synthesis of Asparagine-Linked Glycopeptides on a Polymeric Solid Support**

The current approach to solid phase N-linked glycopeptide synthesis involves construction of a peptide segment
25 bearing a terminal amine equivalent residue on a solid support. The amine is then activated and coupled with either an appropriate oligosaccharide or a smaller glycopeptide. Cleavage from the solid support and deprotection yields the desired glycopeptide (M. Meldal,
30 in *Neoglycoconjugates: Preparation and Applications*, 1994, Y. C. Lee, R. T. Lee, Eds., Academic Press, London; S. J. Danishefsky and J. Y. Roberge, in *Glycopeptides and related compounds: Chemical synthesis, analysis and applications*, 1995, D. G. Large, C. D. Warren, Eds.,
35 Marcel Dekker, New York).

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The method we propose is illustrated in Scheme I (Figure 13). An oligosaccharide terminating in a glycal is constructed on the solid support (see structure 1C). As shown earlier, 1C can be an extended linear structure, or
5 can contain branching as desired (J.T. Randolph, K.F. McClure, S.J. Danishefsky, *J. Amer. Chem. Soc.*, in press; J.T. Randolph and S.J. Danishefsky, *Angew. Chem. Int. Ed. Engl.*, 1994, 33, 1470; S.J. Danishefsky, J.T. Randolph, J.Y. Roberge, K.F. McClure, R.B. Ruggeri, in *The Schering*
10 *Lecture Series* (Germany, 1994), in press; S.J. Danishefsky, J.T. Randolph, J.T. Roberge, K.F. McClure, R.B. Ruggeri, *Polymer Preprint*, 1994, 35, 977.; S.J. Danishefsky, K.F. McClure, J.T. Randolph, R.B. Ruggeri, *Science*, 1993, 260, 1307). Through chemistry described
15 below (Figure 14), 1C is converted to the solid phase bound 2C, bearing a terminal 2-N-acetyl-1 β -aminoglucosamine residue (GlcNAc). A peptide is readily assembled through standard solution phase peptide synthesis methodology or by a solid phase assembly-retrieval
20 sequence. Coupling of 2C with a suitable aspartic acid containing peptide affords solid phase bound glycopeptide 3C. Retrieval and full deprotection affords the desired N-linked glycopeptide. In addition, deprotection of the C-terminus and addition of a peptide with a free N-
25 terminus allows for elongation of the peptide chain while the glycopeptide 3C is still bound to the solid support.

A simple sequence was devised to convert 1C to 2C (Figure 14). The use of the anthracenesulfonamide (A.J. Robinson
30 and P.B. Wyatt, *Tetrahedron*, 1993, 49, 11329) in the azasulfonamidation sequence (F.E. McDonald and S.J. Danishefsky, *J. Org. Chem.*, 1992, 57, 7001) was crucial for the addition step (structure 4C), the azide induced rearrangement (structure 5C) and the presentation of the
35 solid phase bound GlcNAc bearing a 1 β -amino function (structure 2C).

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Two examples of this design for glycopeptide synthesis are illustrated in Figure 15, showing the relative simplicity of protecting group requirements and the high order of convergence of the approach. The two routes
5 respectively afford 22C or 23C after deprotection,

The principle advantage in using the anthracenesulfonamide is that the nitrogen-sulfur linkage can be cleaved by a variety of mild methods (A.J. Robinson and P.B.
10 Wyatt, *Tetrahedron*, 1993, 49, 11329). For instance, the subject invention uses thiophenol or 1,3-propanedithiol and Hunig's base for the removal of the anthracene-sulfonyl group. These protocols are compatible with synthesis on a solid support. Also, anthracenesulfonamide
15 itself is more soluble than benzenesulfonamide in THF, which is a good swelling solvent for the polymer supported steps. Thus, use of the anthracene-based agent results in a more efficient and complete iodosulfonamidation reaction.

20 In solution-phase coupling of carbohydrates and peptides, the process of separating the unreacted components and by-products is not a trivial matter. Purification is greatly simplified by conducting the coupling reaction on
25 the solid support. Most of the excess peptide is recovered by chromatography. Small amounts are lost when the activated aspartic residue cyclizes to an aspartamide. In practice, protected trisaccharide-pentapeptide 21C was 91%. Chromatography on a short
30 column of reverse phase silica (C-18) was sufficient to obtain this compound in pure form. This purification capability arises from the previously described "self-policing" feature of the solid-phase glycal assembly method, which avoids deletions through destruction of
35 uncoupled donors prior to the next coupling cycle (S.J. Danishefsky, K.F. McClure, J.T. Randolph, R.B. Ruggeri, *Science*, 1993, 260, 1307)).

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- The glycopeptides retrieved from the support were deprotected as shown and the fully deblocked glycopeptides 22C and 23C were obtained in 61% and 48% overall yields from 20C and 21C, respectively.
- 5 Structural characterization of the glycopeptides by NMR spectroscopy showed the β configuration of all the anomeric linkages. The structures were further corroborated by mass spectroscopy.
- 10 The presence of orthogonal protecting groups on the C- and N-termini of the peptide provides the opportunity to extend the peptide chain while the ensemble is bound to the solid support. Alternatively, after removal from the support, the freed peptide terminus may provide a
- 15 functionality for linking to a carrier molecule to generate other glycoconjugates (C. Unverzagt and H. Kunz, *Bioorg. Med. Chem.*, 1993, 3, 197)). Figure 16 shows how the peptide portion of the glycopeptide was extended while still bound to the polymer support. Solid phase
- 20 bound trisaccharide pentapeptide 24C was assembled and the C-terminus deprotected as shown to give the acid 25C. Polymer-bound 25C was then coupled to tripeptide 29C with a free N-terminus to give glycopeptide 26C. Retrieval from the solid support afforded trisaccharide-octapeptide
- 25 27C in an 18% overall yield from polymer-bound galactal carbonate.
- The method of the subject invention allows the use of unnatural amino acids and non-amino acids. The method
- 30 is, in principle, totally general in that it does not require the availability of transferases to effect enzymatic coupling or nucleoside activated hexoses. The subject method can further accommodate unnatural (artificial) sugars in the preparation of glycopeptides.
- 35 Such building blocks are available from the Lewis acid catalyzed diene-aldehyde cyclocondensation reaction (S.J. Danishefsky, *Chemtracts*, 1989, 2, 273; D.B. Berkowitz,

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S.J. Danishefsky, G.K. Schulte, *J. Am. Chem. Soc.*, 1992, 114, 4518)). Purely chemical and enzymatic approaches are complementary for preparing fully synthetic glycopeptides.

5

General Methods.

Melting points are not corrected. Infrared spectra were recorded on a Perkin Elmer 1600 series FTIR. ¹H NMR spectra were obtained on a Bruker AMX-400 NMR (400 MHz) and are reported in parts per million (δ) relative to SiMe₄ (0.00 ppm) as an internal reference, with coupling constants (J) reported in hertz. ¹³C NMR spectra were obtained at 100 MHz and are reported in δ relative to CDCl₃ (77.00 ppm) as an internal reference, with coupling constants (J) reported in hertz. High-resolution mass spectra were recorded on a JEOL JMS-DX-303 HF mass spectrometer. Optical rotations were recorded on a Jasco DIP-370 polarimeter using a 1 dm cell at the reported temperatures and concentrations.

Chemicals used were reagent grade and used as supplied except where noted. Pyridine, benzene, and dichloromethane (CH₂Cl₂) were distilled from calcium hydride under N₂. Tetrahydrofuran (THF) was distilled from sodium/benzophenone ketyl under N₂. Analytical thin-layer chromatography was performed on E. Merck silica gel 60 F₂₅₄ plates (0.25 mm) and E. Merck HPTLC RP-18 WF₂₅₄s plates 0.20 mm. Compounds were visualized by dipping the plates in a cerium sulfate-ammonium molybdate solution followed by heating. Liquid column chromatography was performed using forced flow of the indicated solvent on Sigma H-Type silica gel (10-40 mm) for normal phase and EM Science LiChroprep RP-18 (15-25 mm) for reverse phase.

Preparation of the threonine derivative 3C.

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Allyl bromide (11.2 mL, 129 mmol) was added to a suspension of cesium carbonate (8.4 g, 25.9 mmol) and the threonine derivative A (Sigma, 4 g, 12.9 mmol) in 50 mL of DMF. The mixture was stirred 1 h and was poured into 200 mL of water and was extracted with ethyl acetate (3 X 200 mL). The organic extracts were washed with 50 mL of water, 50 mL of brine, dried over sodium sulfate and concentrated. The resulting oil (compound B) was treated with TFA (25 mL) for 10 min. and the solvent was removed under reduced pressure. The oil was diluted with 200 mL of saturated sodium bicarbonate and was extracted with ethyl acetate (3 X 200 mL). The organic extracts were dried with sodium carbonate and concentrated to give C (2.9 g, 100% yield). FTIR (neat); 3388 (NH), 2931, 1739, 1168; ¹H NMR (400 MHz) δ 7.34-7.22 (m, 5H, C₆H₅), 5.9-5.8 (m, 1H, OCH₂CHCH₂), 5.32-5.18 (m, 2H, OCH₂CHCH₂), 4.65-4.3 (m, 4H), 3.98 (dq, 1H, J = 6 Hz, J = 4 Hz, MeCHOBN), 3.40 (d, 1H, J = 4 Hz, Ha Thr), 1.62 (br s, 2H, NH₂), 1.29 (d, 3H, J = 6 Hz, Me); ¹³C δ 173.80, 138.11, 128.13 (2C), 127.47 (2C), 127.41, 118.50, 75.34, 70.67, 65.45, 59.44, 16.21; MS (DCI, NH₃) m/z 250 (MH⁺).

Preparation of the aspartic acid derivative E.

A mixture of 8.2 mL of aqueous sodium hydroxide (1 M, 8.2 mmol) and 16.4 mL of aqueous sodium bicarbonate (1 M, 16.4 mmol) was added to the b-(p-methoxybenzoate) aspartic acid D (2.1 g, 8.2 mmol). (W.A.R. van Heeswijk, et al., *Synthesis*, 1982, 744) 16 mL of dioxane was added to dissolve the white paste and 2,2,2-trichloroethyl chloroformate (TrocCl, 1.24 mL, 9.03 mmol) was slowly added. Evolution of gas was observed and the mixture was stirred for 6 h. The solution was then extracted with ethyl acetate (3 X 100 mL), the aqueous layer was acidified with concentrated HCl and was extracted with ethyl acetate (2 X 100 mL). The organic

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extracts were combined, washed with 100 mL of brine, dried with sodium sulfate and the solvents were removed under reduced pressure. The residue was purified on silica [100 g, 80:19:1 CH₂Cl₂:acetone:acetic acid → 10:29:1] to give E (2.63 g, 75% yield). R_f = 0.3 [80:19:1 CH₂Cl₂:acetone:acetic acid]; FTIR (neat); 3327 (OH), 2958, 1732, 1516, 1248; ¹H NMR (400 MHz) δ 10-9 (br s, 1H, COOH), 7.28 (d, 2H, J = 8 Hz, PMB), 6.89 (d, 2H, J = 8 Hz, PMB), 6.01 (br d, 2H, J = 8 Hz, NH), 5.15-5.05 (m, 2H, PMB), 4.8-4.6 (m, 3H), 3.81 (s, 3H, OMe), 3.13 (dd, 1H, J = 17 Hz, J = 4 Hz, Hb Asn), 2.93 (dd, 1H, J = 17 Hz, J = 5 Hz, Hb Asn); ¹³C δ 175.12, 170.72, 159.63, 154.35, 130.11 (2C), 127.07, 113.92 (2C), 95.05, 74.65, 66.95, 55.14, 50.26, 36.16; MS (DCI, NH₃) m/z 427 (M+).

15

Preparation of the dipeptide F.

A suspension of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDACI, 862 mg, 4.5 mmol) in 4 mL of DMF was added to a mixture of the *p*-toluenesulfonyl salt of leucine allyl ester (Sigma, 1.54 g, 4.5 mmol), *N*-methymorpholine (494 mL, 4.5 mmol) and 1-hydroxybenzotriazole (HOBt, 608 mg, 4.5 mmol) in 10 mL of CH₂Cl₂. The mixture was stirred for 30 h and was then diluted with 200 mL of ethyl acetate, washed with 50 mL of saturated sodium bicarbonate, 50 mL of 2 *N* citric acid, 30 mL of saturated sodium bicarbonate, 30 mL of water and 30 mL of brine. The organic extracts were dried with sodium sulfate and the solvents were removed under reduced pressure. The residue was purified on silica [60 g, 7:3 hexane:ethyl acetate] to give F (2.11 g, 97% yield). R_f = 0.4 [7:3 hexane:ethyl acetate]; FTIR (neat); 3329 (NH), 2957, 1740, 1670 (C=O), 1516, 1248; ¹H NMR (400 MHz) δ 7.29 (d, 2H, J = 8 Hz, PMB), 6.95-6.85 (m, 3H, NH, PMB), 6.21 (br d, 2H, J = 8 Hz, NH), 5.95-5.8 (m, 1H, OCH₂CHCH₂), 5.35-5.23 (m, 2H, OCH₂CHCH₂), 5.10 (s, 2H, PMB), 4.8-4.5 (m, 6H), 3.81 (s, 3H, OMe), 3.07 (dd,

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1H, $J = 17$ Hz, $J = 6$ Hz, Hb Asn), 2.75 (dd, 1H, $J = 17$ Hz, $J = 6$ Hz, Hb Asn), 1.75-1.5 (m, 3H, Hb, Hg Leu), 0.95 (d, 3H, $J = 6$ Hz, Me Leu), 0.93 (d, 3H, $J = 6$ Hz, Me Leu); ^{13}C d 171.95, 171.83, 169.61, 159.77, 154.12, 131.49, 130.17 (2C), 127.20, 118.81, 113.97 (2C), 95.19, 74.67, 66.98, 65.89, 55.24, 51.08, 50.83, 41.18, 36.42, 24.77, 22.77, 21.77; MS (DCI, NH_3) m/z 581 (MH+).

Preparation of the dipeptide G.

Morpholine (300 mL, 3.4 mmol) and tetrakis(triphenylphosphine) palladium (20 mg, 0.017 mmol) were successively added to the dipeptide F (200 mg, 0.34 mmol), dissolved in 3 mL of THF, and the dark orange solution was stirred at room temperature for 4 h. (H. Waldmann and H. Kunz, *Liebigs Ann. Chem.*, 1983, 1712) The mixture was poured into 15 mL of aqueous HCl 1 M, extracted with 125 mL of ethyl acetate, washed with 15 mL of water, 20 mL of brine and dried over sodium sulfate. The solvent were removed under reduced pressure and the residue was dissolved in a minimum amount of MeOH (0.3 mL) and applied over reverse phase silica [6 g, C-18, 1:1 H_2O (0.1 % TFA):MeOH \rightarrow MeOH] to give G (157 mg, 85% yield). $R_f = 0.4$ [RP-18, 3:7 H_2O (0.1 % TFA):MeOH]; FTIR (neat); 3315 (OH), 2958, 1723, 1669 (C=O), 1515, 1247; ^1H NMR (400 MHz) d 10.1-9.6 (br s, 1H, COOH), 7.27 (d, 2H, $J = 8$ Hz, PMB), 6.97 (br d, 1H, $J = 8$ Hz, NH), 6.88 (d, 2H, $J = 8$ Hz, PMB), 6.36 (br d, 1H, $J = 9$ Hz, NH), 5.11 (s, 2H, PMB), 4.8-4.5 (m, 4H), 3.80 (s, 3H, OMe), 3.00 (dd, 1H, $J = 17$ Hz, $J = 6$ Hz, Hb Asn), 2.77 (dd, 1H, $J = 17$ Hz, $J = 6$ Hz, Hb Asn), 1.75-1.5 (m, 3H, Hb, Hg Leu), 0.95 (d, 3H, $J = 6$ Hz, Me Leu), 0.93 (d, 3H, $J = 6$ Hz, Me Leu); ^{13}C d 176.65, 171.74, 170.23, 159.75, 154.31, 130.18 (2C), 127.16, 113.98 (2C), 95.16, 74.69, 67.05, 55.24, 51.00, 50.96, 40.77, 36.37, 24.77, 22.78, 21.64; MS (DCI, NH_3) m/z 541 (MH+).

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Preparation of the tripeptide 16AC.

2-Isobutoxy-1-isobutoxycarbonyl-1,2-dihydroquinoline (IIDQ, 267 mL, 0.9 mmol) was added to a mixture of the dipeptide G (424 mg, -0.78 mmol) and the threonine derivative C (234 mg, 0.94 mmol) in 8 mL of CH₂Cl₂. The mixture was stirred 4 h at room temperature and was poured into 25 mL of 0.05 N aqueous HCl. The mixture was extracted with ethyl acetate (3 X 25 mL). The combined organic extracts were washed with 20 mL of water and 20 mL of brine and dried over sodium sulfate. The solvents were removed under reduced pressure and the resulting oil was purified by chromatography (silica, 20 g, 7:3 hexane:ethyl acetate) to give 16AC (555 mg, 92% yield) as a white solid. R_f = 0.45 [3:2 hexane:ethyl acetate]; FTIR (KBr pellet); 3285 (OH), 3086, 2956, 1739, 1651 (C=O), 1538, 1207; ¹H NMR (400 MHz) δ 7.35-7.2 (m, 7H, C₆H₅, PMB), 6.9-6.85 (m, 3H, PMB, NH), 6.52 (br d, 1H, J = 9 Hz, NH), 6.17 (br d, 1H, J = 9 Hz, NH), 5.9-5.75 (m, 1H, OCH₂CHCH₂), 5.30-5.20 (m, 1H, OCH₂CHCH₂), 5.05 (s, 2H, PMB), 4.7-4.1 (m, 10H), 3.80 (s, 3H, OMe), 3.02 (dd, 1H, J = 17 Hz, J = 6 Hz, Hb Asn), 2.73 (dd, 1H, J = 17 Hz, J = 6 Hz, Hb Asn), 1.75-1.5 (m, 3H, Hb, Hg Leu), 1.18 (d, 3H, J = 6 Hz, Me Thr), 0.95 (d, 3H, J = 6 Hz, Me Leu), 0.93 (d, 3H, J = 6 Hz, Me Leu); ¹³C δ 171.86, 171.71, 169.90, 169.63, 159.78, 154.15, 137.68, 131.47, 130.20, 128.36 (2C), 127.82 (3C), 127.79 (2C), 127.24, 119.04, 113.99 (2C), 95.25, 74.72, 74.02, 70.75, 66.98, 66.11, 56.68, 52.27, 52.15, 51.02, 41.30, 36.32, 24.64, 22.89, 22.00, 16.14.

Preparation of the tripeptide 16C.

TFA (5 mL) was added to the tripeptide 16AC (553 mg, -0.72 mmol) at 0 °C, the purple solution was stirred 5 min. at room temperature and the TFA was removed under reduced pressure. The oil was purified by chromatography

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(silica, 20 g, 98:2 CH₂Cl₂:MeOH → 95:5) to give 16C (349 mg, 75% yield) as a white solid. R_f = 0.4 [95:5 CH₂Cl₂:MeOH]; FTIR (KBr pellet); 3285 (OH), 3086, 2956, 1739, 1651 (C=O), 1538, 1207; ¹H NMR (400 MHz) δ 9.2-8.5 (br s, 1H, COOH), 7.4-7.2 (m, 6H, C₆H₅, NH), 7.05 (br d, 1H, J = 9 Hz, NH), 6.39 (br d, 1H, J = 9 Hz, NH), 5.85-5.70 (m, 1H, OCH₂CHCH₂), 5.30-5.20 (m, 1H, OCH₂CHCH₂), 4.8-4.1 (m, 10H), 2.95 (dd, 1H, J = 17 Hz, J = 6 Hz, Hb Asn), 2.78 (dd, 1H, J = 17 Hz, J = 6 Hz, Hb Asn), 1.75-1.5 (m, 3H, Hb, Hg Leu), 1.18 (d, 3H, J = 6 Hz, Me Thr), 0.95 (d, 3H, J = 6 Hz, Me Leu), 0.91 (d, 3H, J = 6 Hz, Me Leu); ¹³C δ 173.85, 173.28, 170.25, 169.65, 154.15, 137.25, 131.32, 128.38 (2C), 127.95 (3C), 119.15, 95.28, 74.60, 74.09, 70.90, 66.21, 56.78, 52.09, 51.51, 41.07, 36.21, 24.56, 22.55, 22.29, 15.92; MS (DCI, NH₃) m/z 652 (MH⁺).

Preparation of the polymer-bound trisaccharide-glycal 11X.

In a polymer synthesis flask, the polymer-bound disaccharide-glycal 10X (981 mg, ~0.77 mmol) was suspended in 15 mL of CH₂Cl₂ for 1 h at room temperature. The mixture was cooled to 0 °C and 3,3-dimethyldioxirane (30 mL in acetone, ~2.4 mmol) was added and the mixture was gently stirred for 1 h 20. The solvents were filtered and the resin was kept under reduced pressure for 7 h. A solution of 3,4-dibenzyl glucal (dried by azeotropic distillation with benzene, 1.67 g, 5.12 mmol) in 10 mL of THF was added at 0 °C to the polymer-bound epoxide and a solution of zinc chloride (1.53 mL, 1 M in ether, 1.53 mmol). The mixture was gently stirred at room temperature for 16 h. The solvents were filtered, the resin was successively washed with THF (4 X 50 mL) and CH₂Cl₂ (3 X 50 mL) and was kept

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Preparation of the polymer-bound trisaccharide-glycal 9C.

5 In a polymer synthesis flask, the polymer-bound trisaccharide-glycal 11X (1.23 g, ~0.76 mmol) was suspended in 20 mL of THF for 1 h at room temperature. Acetic anhydride (1.44 mL, 15.2 mmol) and *sym*-collidine (2.0 mL, 15.2 mmol) were successively added and the
10 mixture was gently stirred for 12 h. The solvents were filtered, the resin was successively washed with THF (4 X 50 mL) and CH₂Cl₂ (3 X 50 mL) and was kept under vacuum overnight to give 1.22 g of polymer bound trisaccharide 9C.

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Preparation of the polymer-bound iodo-sulfonamide 11C.

In a polymer synthesis flask, the polymer-bound trisaccharide-glycal 9C (559 mg, ~0.212 mmol) and 9-anthracenesulfonamide (381 mg, 1.5 mmol) were suspended
20 in 15 mL of THF for 1 h at room temperature. The mixture was cooled to -10 °C (ice-acetone bath) and freshly prepared iodonium bis(*sym*-collidine) perchlorate (261 mg, 1.1 mmol) was added in one portion and the mixture was
25 gently stirred for 10 min. at this temperature. The yellow suspension was warmed to 0 °C and stirred for an additional hour before being cooled to -10 °C. A cold (-10 °C) solution ascorbic acid (2 g) in THF:water (10:1, 40 mL) was added, turning the solution almost colorless
30 while the polymer remained bright yellow. After 5 min. at -10 °C, the mixture was warm to room temperature (~1 h). The solvents were filtered, the resin was successively washed with THF (4 X 50 mL) and CH₂Cl₂ (3 X 50 mL) and was kept under vacuum overnight to give 643 mg
35 of polymer bound trisaccharide 11C. This resin fluoresce blue at 365 nm

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Iodonium bis(sym-collidine) perchlorate: Under nitrogen, iodine (775 mg, 3 mmol) was added to a suspension of silver bis(sym-collidine) perchlorate and sym-collidine (45 mL, 0.34 mmol) in 12 mL of chloroform (dried over activated basic alumina). The mixture was vigorously stirred for 15 min. and the bright yellow suspension was filtered over flame-dried Celite[™] under dry nitrogen. Ether (10 mL) were added and the faintly green precipitate was filtered over sintered glass under dried nitrogen, washed with ether and dried under reduced pressure. Yield ~1g.

Preparation of the polymer-bound trisaccharide-azide 12C.

In a polymer synthesis flask, the polymer-bound iodo-sulfonamide 11C (553 mg, ~0.18 mmol) was suspended in 12 mL of THF for 1 h at room temperature. Tetrabutylammonium azide (261 mg, 1.1 mmol) was added and the mixture was gently stirred for 5 h. The solvent was filtered, the resin was successively washed with THF (4 X 50 mL) and CH₂Cl₂ (3 X 50 mL) and was kept under vacuum overnight to give 520 mg of polymer bound trisaccharide 12C. This resin also fluoresce blue at 365 nm.

Preparation of the polymer-bound trisaccharide-azide 13C.

In a polymer synthesis flask, the polymer-bound azide-sulfonamide 12C (469 mg, ~0.16 mmol) was suspended in 15 mL of THF for 1 h at room temperature. Acetic anhydride (752 mL, 8 mmol) and 4-N,N-dimethylaminopyridine (779 mg, 6.4 mmol) were successively added and the mixture was gently stirred for 3 h. The solvent was filtered, the resin was successively washed with THF (3 X 50 mL) and CH₂Cl₂ (3 X 50 mL) and was kept under vacuum overnight to give 480 mg of polymer bound trisaccharide 13C. This resin fluoresces green at 365 nm.

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Preparation of the polymer-bound trisaccharide-amine 14C.

In a polymer synthesis flask, the polymer-bound trisaccharide-azide-sulfonamide 13C (51 mg, ~0.018 mmol) was suspended in 5 mL of DMF for 1 h at room temperature. 1,3-Propanedithiol (105 mL, 1.04 mmol) and *N,N*-diisopropyl-*N*-ethylamine (109 mL, 0.63 mmol) were added and the suspension was gently stirred at room temperature for 6 h. The resin was successively washed with DMF (3 mL) and THF (2 X 4 mL) and an aliquot of the resin was examined by IR (KBr pellet). The reaction was incomplete as was indicated by the presence of an azide stretch at 2115 cm^{-1} . The resin was suspended in 2 mL of DMF and 1,3-propanedithiol (220 mL, 2.19 mmol) and *N,N*-diisopropyl-*N*-ethylamine (220 mL, 1.26 mmol) were added and the suspension was gently stirred at room temperature for 12 h. The resin was successively washed with DMF (3 mL), THF (2 X 5 mL) and CH_2Cl_2 (2 X 5 mL). The resin was dried under reduced pressure to give 51 mg of solid 14C. This resin did not substantially fluoresce at 365 nm.

Preparation of the polymer-bound trisaccharide-tripeptide 18C.

In a polymer synthesis flask, the polymer-bound trisaccharide-amine 14C (51 mg, ~0.018 mmol) was suspended in 1.5 mL of CH_2Cl_2 for 1 h at room temperature. The tripeptide 16C (24 mg, 0.37 mmol) and IIDQ (Aldrich, 10 mL, 0.034 mmol) were successively added. After 18 h, the solvent was filtered and the resin was washed sequentially with CH_2Cl_2 (3 X 5 mL), THF (5 mL), CH_2Cl_2 (5 mL) and the resin was dried under reduced pressure to give 57 mg of resin 18C.

Preparation of the trisaccharide-tripeptide 20C.

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In a Teflon™ flask, the polymer-bound trisaccharide-tripeptide 18C (57 mg, ~0.018 mmol) and anisole (7.4 mL, 0.68 mmol) were suspended in 6 mL of CH₂Cl₂ for 1 h at room temperature. The mixture was cooled to -10°C (acetone/ice) and HF·pyridine (~50 mL, 1.7 mmol) was slowly added. After 2 h, H₂O (5 mL) was added at -10°C and the mixture was extracted with ethyl acetate (3 X 30 mL). The combined organic layers were washed twice with a mixture of brine (10 mL) and saturated NaHCO₃ (1-2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter and concentrated. The residue was purified over silica [1.5 g, 99:1 CH₂Cl₂:MeOH → 95:5] to give 20C slightly impure (7.8 mg, ~31%), R_f = 0.26 [95:5 CH₂Cl₂:MeOH]. Further attempt to purify on silica gel led to decomposition. The product could be carried through the deprotection sequence in greater overall yield if the initial purification is omitted.

20 Preparation of the trisaccharide-tripeptide 20X.

Dimethylbarbituric acid (17 mg, 0.11 mmol) and tetrakis(triphenylphosphine) palladium (2 mg, 0.0018 mmol) were successively added to a solution of alcohol 20C (27.2 mg, 0.018 mmol) in 2.5 mL of THF and the dark orange mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of MeOH (~0.3 mL) and applied over reverse phase silica [3 g, C-18, 1:1 H₂O (0.1 % TFA):MeOH → MeOH] to give 20X (29 mg). The product was contaminated with byproducts of the tetrakis(triphenylphosphine) palladium and was used for the next step.

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Preparation of the trisaccharide-tripeptide 20X.

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The acid 20X (26.2 mg, 0.018 mmol) was dissolved in a mixture of 2.5 mL of MeOH and 0.6 mL of acetic acid and zinc dust (Aldrich, 30 mg, 0.45 mmol) was added. The suspension was stirred at room temperature for 15 h. The gray mixture was filtered over Celite™ and the solids were washed successively with ethanol (15 mL) and MeOH (15 mL) the solvents were combined and evaporated. The residue was applied on reverse phase silica [3 g, C-18, 7:3 H₂O (0.1 % TFA):MeOH → 9:1] to give 20X as its TFA salt (20.7 mg, ~83%). $R_f = 0.23$ [7:3 H₂O (0.1 % TFA):MeOH]; FTIR (KBr pellet); 3306 (OH), 2928, 1804, 1752, 1670 (C=O), 1534, 1372, 1083; The product 20X was still slightly impure by ¹H NMR and was used for the next step.

Synthesis of the trisaccharide-tripeptide 22C.

Compound 20X (20.7 mg, 0.014 mmol) was dissolved in 2 mL of MeOH and 2 mL of acetic acid and palladium(II) acetate (12 mg, 0.053 mmol) was added. The yellow mixture was kept under a positive pressure of hydrogen (balloon) for 17 h with efficient stirring. At the end of this period, the black suspension was sonicated for 5 min., filtered over Celite™ and the solids were washed with MeOH and acetic acid. The solvents were removed under reduced pressure. The completion of the reaction was monitored by observing the disappearance of the aromatic signals in the crude ¹H NMR spectra of the product in D₂O. The solvent was removed under reduced pressure and the residue was dissolved 4 mL of MeOH. Potassium cyanide (2.6 mg, 0.04 mmol) was added, the pH was observed to be between 7 and 8 (moist pH paper). After 1h, additional potassium cyanide was added (1 mg, 0.015 mmol) and the mixture was stirred at pH ~8 for 9 h. 100 mL of Acetic acid was added, most of the solvents were removed under a flow of nitrogen in an efficient fume hood and the residue was kept under vacuum for 30 min. The glassy

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solid was applied on reverse phase silica [1 g, C-18, H₂O (0.1 % TFA) → 9:1 H₂O (0.1 % TFA):MeOH] to give 22C as its TFA salt (10 mg, 65% overall yield from 22C). $R_f = 0.55$ [H₂O (0.1 % TFA)]; $[\alpha]_D^{23} = -0.6^\circ$ (c 0.48, H₂O); FTIR (KBr pellet); 3424 (OH), 2928, 1653 (C=O), 1552, 1388, 1077; ¹H NMR (400 MHz, D₂O) δ 5.10 (d, 1H, $J = 10$ Hz, H1 GlcNAc), 4.46 (d, 1H, $J = 9$ Hz, H1 Gal), 4.44 (d, 1H, $J = 8$ Hz, H1 Gal), 4.39 (dd, 1H, $J = 9$ Hz, $J = 4$ Hz, Ha Asn), 4.30-4.22 (m, 2H, Hb Thr, Ha Leu), 4.10-4.03 (m, 1H), 4.00-3.50 (m, 18H), 3.05 (dd, 1H, $J = 18$ Hz, $J = 4$ Hz, Hb Asn), 2.90 (dd, 1H, $J = 18$ Hz, $J = 9$ Hz, Hb Asn), 2.01 (s, 3H, NHAc), 1.75-1.63 (m, 3H, Hb, Hg Leu), 1.17 (d 3H, $J = 6$ Hz, Me Thr), 0.95 (d 3H, $J = 6$ Hz, Me Leu), 0.91 (d 3H, $J = 6$ Hz, Me Leu); ¹³C (D₂O) δ 177.40, 176.21, 175.21, 172.57, 169.96, 164.90 (² $J_{CF} = 37$ Hz), 115.50 (¹ $J_{CF} = 286$ Hz), 104.71, 104.63, 79.72, 77.95, 76.58, 75.61, 75.33, 74.11, 73.91, 72.15, 72.08, 70.81, 70.39, 70.10, 70.06, 70.02, 69.37, 62.39, 61.46, 55.35, 54.51, 50.88, 41.05, 36.77, 25.75, 23.52 (2C), 22.12, 20.60; HRMS (FAB, Na+) m/z 874.3821 (MH+), calcd for C₃₄H₆₀N₅O₂₁ 874.3780.

POLYMER-SUPPORTED SYNTHESIS OF TRISACCHARIDE PENTAPEPTIDE

In a polymer synthesis flask, the polymer-bound trisaccharide 14C (120.9 mg, ~0.014 mmol) was swollen in 4 mL of CH₂Cl₂ for 1 h at room temperature. The pentapeptide CbzAlaLeuAspLeuThr(OBn)OAll (96.4 mg, 0.106 mmol) and IIDQ (Aldrich, 30 mL, 0.101 mmol) were successively added. After 25 h, the solvent was filtered and the resin was washed with CH₂Cl₂ (5 X 5 mL), THF (1 X 5 mL), CH₂Cl₂ (3 X 5 mL) and the resin was dried under reduced pressure to give 133.3 mg of resin 30C.

In a Teflon™ flask, the polymer-bound trisaccharide-pentapeptide 30C (94.3 mg, ~0.0273 mmol) and anisole (8.5

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- mL, 0.78 mmol) were suspended in 5 mL of CH_2Cl_2 for 1 h at room temperature. The mixture was cooled to -10°C (acetone/ice) and $\text{HF}\cdot\text{pyridine}$ (~50 mL, 1.7 mmol) was slowly added. After 2 h, H_2O (5 mL) was added at -10°C and the mixture was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were washed twice with a mixture of brine (10 mL) and saturated NaHCO_3 (1-2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter and concentrated. The residue was purified over RP-18 silica, eluting with 7:3 $\text{MeOH}:\text{H}_2\text{O}$ (0.1 % TFA) \rightarrow 9:1 to give trisaccharie-pentapeptide 31C (17.9 mg, -37%): $[\alpha]^{24}_{\text{D}} -14.2^\circ$ (c 1.38, CH_2Cl_2); ^1H NMR (400 MHz, CD_3OD) δ 7.31 (20H, m), 5.88, (1H, ddd, $J = 5.5, 10.5, 16.5$ Hz), 5.31 (1H, dd, $J = 1.0, 16.5$ Hz), 5.19 (1H, d, $J = 1.2, 10.5$ Hz), 5.14-5.00 (5H, m), 4.96 (1H, t, $J = 4.0$ Hz), 4.92-4.89 (6H, m), 4.81-4.68 (7H, m), 4.66-4.57 (7H, m), 4.49-4.41 (2H, m), 4.34 (1H, d, $J = 6.0, 9.0$ Hz), 4.19-4.00 (7H, m), 3.92 (1H, t, $J = 10.0$ Hz), 3.82-3.67 (6H, m), 3.52 (2H, d, $J = 5.0$ Hz), 2.76 (1H, d, $J = 5.0, 16.0$ Hz), 2.64 (1H, dd, $J = 7.0, 16.0$ Hz), 2.09 (3H, s), 2.06 (3H, s), 1.85 (3H, s), 1.74-1.60 (6H, m), 1.35 (3H, d, $J = 7.0$ Hz), 1.22 (3H, d, $J = 6.5$ Hz), 0.94 (6H, d, $J = 6.0$ Hz), 0.89 (6H, d, $J = 6.0$ Hz); ^{13}C NMR (CD_3OD) δ 175.6, 174.9, 174.3, 173.6, 172.5, 172.4, 171.2, 170.7, 168.8, 161.8, 155.6, 155.4, 139.7, 139.4, 139.3, 133.1, 129.4, 129.3, 128.9, 128.8, 128.7, 128.6, 119.1, 102.0, 99.2, 84.4, 79.7, 79.1, 76.6, 76.1, 75.8, 75.6, 75.4, 73.3, 71.9, 71.2, 70.6, 69.8, 68.6, 68.4, 68.3, 67.7, 67.0, 61.8, 58.2, 55.5, 53.3, 53.2, 52.1, 51.1, 41.9, 41.4, 37.8, 25.8, 25.6, 23.5, 23.2, 22.1, 21.9, 21.2, 20.8, 18.1, 16.5; HRMS (FAB) calcd for $\text{C}_{81}\text{H}_{103}\text{N}_7\text{O}_{29}$ 1638.6881, found 1638.6840.
- Dimethylbarbituric acid (2.1 mg, 0.013 mmol) and tetra-kis(triphenylphosphine) palladium (1.0 mg, 0.0009 mmol) were successively added to a solution of alcohol 31C (3.6

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mg, 0.0022 mmol) in 2.0 mL of THF and the dark orange mixture was stirred at room temperature for 5 h. The solvent was removed under reduced pressure and the residue was dissolved in a minimum amount of MeOH (~0.3 mL) and applied over reverse phase silica, C-18, 7:3 H₂O (0.1 % TFA):MeOH → MeOH] to give trisaccharide-pentapeptide 32C (3.5 mg, 100%): $[\alpha]^{24}_D -14.3^\circ$ (c 0.46, CH₂Cl₂); FTIR (neat) 3298, 1812, 1754, 1651, 1514, 1454, 1371, 1221, 1168, 1069 cm⁻¹; ¹H NMR (400 MHz, CD₃OD) δ 8.25 (1H, d, J = 9.0 Hz), 8.16 (1H, d, J = 8.0 Hz), 8.07 (1H, d, J = 7.5 Hz), 7.91-7.87 (2H, m), 7.56-7.51 (2H, m), 7.31 (20H, m), 5.13-4.99 (4H, m), 4.96 (1H, t, J = 3.5 Hz), 4.91-4.89 (5H, m), 4.79-4.69 (5H, m), 4.66-4.60 (3H, m), 4.57-4.54 (1H, m), 4.50-4.45 (2H, m), 4.31-4.38 (1H, m), 4.21-4.00 (6H, m), 3.92 (1H, t, J = 10.0 Hz), 3.86-3.64 (6H, m), 3.52 (2H, d, J = 5.5 Hz), 2.75 (1H, dd, J = 5.0, 16.0 Hz), 2.67 (1H, dd, J = 9.0, 16.0 Hz), 2.09 (3H, s), 2.05 (3H, s), 1.87 (3H, s), 1.73-1.57 (6H, m), 1.35 (3H, d, J = 7.0 Hz), 1.21 (3H, d, J = 6.5 Hz), 0.94 (6H, d, J = 6.0 Hz), 0.89 (6H, d, J = 6.0 Hz); ¹³C NMR (CD₃OD) δ 199.2, 175.8, 174.9, 174.0, 173.4, 172.7, 172.6, 170.9, 155.8, 155.6, 139.9, 139.6, 129.5, 129.4, 129.1, 129.0, 128.9, 128.8, 128.7, 100.1, 99.5, 84.8, 79.9, 79.3, 77.4, 76.9, 76.1, 75.8, 75.6, 75.2, 75.0, 72.3, 72.2, 71.8, 71.4, 70.1, 68.9, 68.6, 67.8, 62.0, 58.1, 55.6, 53.4, 52.2, 51.3, 41.9, 41.6, 37.9, 25.9, 25.7, 23.6, 23.2, 22.1, 21.9, 20.8, 18.2, 16.7.

In a sealed tube, a mixture of Pd(OAc)₂ and glycopeptide 32C (4.7 mg, 0.0027 mmol) in 4 mL of CH₃OH and 2 drops of acetic acid was charged and evacuated with 4 x H₂ and filled to a pressure of 60 psi and stirred overnight. The reaction was then sonicated, filtered through celite, and the solvent removed in vacuo to give a white powder, the crude ¹H NMR of which showed more benzyl groups in the aromatic region.

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- The debenzylated glycopeptide was redissolved in 1 mL of CH₃OH and 50 mL of 0.1 M KCN/CH₃OH was added. The reaction was pH 8-9 by pH paper. It was stirred at room temperature for 4 hr, then quenched with 2 drops of trifluoroacetic acid and the solvent removed under a stream of N₂. The crude material was purified by RP-18 column chromatography, eluting with a gradient of 1:9 to 9:1 CH₃OH:H₂O to give 1.2 mg (0.0010 mmol, 37%) of the glycopeptide 33C as a white powder: $[\alpha]_D^{24}$ (c); FTIR (neat) 3326, 1671, 1538, 1204 cm⁻¹; ¹H NMR (400 MHz, D₂O) δ 5.08 (1H, d, J = 9.8 Hz), 4.75 (1H, m), 4.49 (1H, d, J = 7.8 Hz), 4.45 (1H, d, J = 7.9 Hz), 4.40-4.24 (3H, m), 4.07 (1H, dd, J = 3.0, 9.5 Hz), 3.98-3.52 (11H, m), 2.90 (1H, dd, J = 5.5, 16.5 Hz), 2.77 (dd, J = 7.5, 16.5 Hz), 2.03 (3H, s), 1.70-1.56 (6H, m), 1.39 (3H, d, J = 7.0 Hz), 1.20 (3H, d, J = 6.5 Hz), 0.96 (6H, d, J = 6.0 Hz), 0.90 (6H, d, J = 6.0 Hz); HRMS (FAB) calcd for C₄₃H₇₅N₇O₂₃Na 1080.4792, found 1080.4800.
- 20 In a polymer synthesis flask, the polymer-bound trisaccharide 14C (199.6 mg, ~0.060 mmol) was swollen in 4 mL of CH₂Cl₂ for 1 h at room temperature. The pentapeptide CbzAlaLeuAspLeuSer(OBn)OAll (76.3 mg, 0.0976 mmol) and IIDQ (Aldrich, 28 mL, 0.0943 mmol) were successively added. After 23 h, the solvent was filtered and the resin was washed with CH₂Cl₂ (4 X 5 mL) and the resin was dried under reduced pressure to give 225.6 mg of resin 34C.
- 30 In a Teflon[®] flask, the polymer-bound trisaccharide-pentapeptide 34C (100.2 mg, ~0.0303 mmol) and anisole (8.5 mL, 0.78 mmol) were suspended in 5 mL of CH₂Cl₂ for 1 h at room temperature. The mixture was cooled to -10°C (acetone/ice) and HF·pyridine (~60 mL, 2.0 mmol) was slowly added. After 2 h, H₂O (5 mL) was added at -10°C and the mixture was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were washed twice with

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a mixture of brine (10 mL) and saturated NaHCO_3 (1-2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter and concentrated. The residue was purified over RP-18 silica, eluting with 7:3 $\text{MeOH:H}_2\text{O}$ (0.1 % TFA) → 9:1 to give trisaccharide-pentapeptide 35C (11.4 mg, ~23%): $[\alpha]_D^{24}$ -14.4° (c 1.09, CH_2Cl_2); ^1H NMR (400 MHz, CD_3OD) δ 8.20-8.07 (3H, m), 7.99 (1H, d, J = 6.5 Hz), 7.94-7.88 (2H, m), 7.82 (1H, d, J = 8.0 Hz), 7.31 (20H, m), 5.90, (1H, ddd, J = 5.5, 10.5, 16.5 Hz), 5.32 (1H, dd, J = 1.5, 16.5 Hz), 5.21 (1H, d, J = 10.5 Hz), 5.15-4.87 (11H, m), 4.78-4.45 (11H, m), 4.33 (1H, m), 4.15-4.00 (6H, m), 3.89 (2H, m), 3.84-3.67 (7H, m), 3.54 (2H, m), 2.79 (1H, d, J = 5.5, 16.0 Hz), 2.63 (1H, dd, J = 6.0, 16.0 Hz), 2.10 (3H, s), 2.08 (3H, s), 1.86 (3H, s), 1.70-1.60 (6H, m), 1.37 (3H, d, J = 7.0 Hz), 0.95 (6H, d, J = 6.0 Hz), 0.90 (6H, d, J = 5.0 Hz); ^{13}C NMR (CD_3OD) δ 175.5, 174.3, 174.2, 173.6, 172.2, 170.7, 170.5, 155.7, 155.3, 139.4, 139.2, 138.7, 132.8, 129.3, 129.2, 128.8, 128.7, 128.6, 128.5, 118.8, 99.5, 98.9, 84.2, 79.5, 79.0, 77.1, 76.1, 75.8, 75.6, 75.1, 74.5, 74.0, 71.6, 71.2, 70.7, 70.1, 69.5, 68.4, 68.3, 7.7, 66.9, 61.7, 55.2, 53.9, 53.3, 52.9, 51.9, 41.5, 41.2, 37.7, 25.6, 25.4, 23.5, 23.4, 23.1, 21.9, 21.8, 20.8, 18.1; HRMS (FAB) calcd for $\text{C}_{80}\text{H}_{101}\text{N}_7\text{O}_{29}\text{Na}$ 1646.6514, found 1646.6530.

In a polymer synthesis flask, the polymer-bound trisaccharide-pentapeptide 34C (125.4 mg, ~0.030 mmol) was swollen in 6 mL of THF for 1 h at room temperature. To this mixture, dimethylbarbituric acid (140 mg, 0.90 mmol) and tetrakis (triphenylphosphine) palladium (17 mg, 0.015 mmol) were successively added and the dark orange mixture was stirred at room temperature. After 14 h, the solvent was filtered and the resin was washed with CH_2Cl_2 (4 X 10 mL) and the resin was dried under reduced pressure to give 113.5 mg of resin 36C.

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In a Teflon[™] flask, the polymer-bound trisaccharide-pentapeptide 36C (98.9 mg, ~0.0301 mmol) and anisole (8.5 mL, 0.78 mmol) were suspended in 5 mL of CH₂Cl₂ for 1 h at room temperature. The mixture was cooled to -10°C (acetone/ice) and HF·pyridine (~60 mL, 2.0 mmol) was slowly added. After 2 h, H₂O (5 mL) was added at -10°C and the mixture was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were washed twice with a mixture of brine (10 mL) and saturated NaHCO₃ (1-2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter and concentrated. The residue was purified over RP-18 silica, eluting with 7:3 MeOH:H₂O(0.1 % TFA) → 9:1 to give trisaccharide-pentapeptide 37C (9.2 mg, ~19%): [α]_D²⁴ -10.1° (c 0.49, CH₂Cl₂); ¹H NMR (400 MHz, CD₃OD) δ 8.17 (1H, d, J = 8.0 Hz), 8.07 (1H, d, J = 7.5 Hz), 7.92 (1H, d, J = 8.0 Hz), 7.49 (1H, m), 7.30 (20H, m), 5.11-4.90 (11H, m), 4.79-4.44 (10H, m), 4.34 (1H, m), 4.16-4.00 (7H, m), 3.94-3.87 (2H, m), 3.82-3.71 (8H, m), 3.52 (2H, d, J = 5.5 Hz), 2.97 (1H, dd, J = 6.0, 16.0 Hz), 2.64 (1H, dd, J = 6.5, 16.0 Hz), 2.09 (3H, s), 2.06 (3H, s), 1.85 (3H, s), 1.70-1.58 (6H, m), 1.35 (3H, d, J = 7.0 Hz), 0.94 (6H, d, J = 6.0 Hz), 0.89 (6H, d, J = 6.0 Hz).

25 Preparation of the polymer-bound trisaccharide-octapeptide.

In a polymer synthesis flask, the polymer-bound trisaccharide-pentapeptide 36C (113.5 mg, ~0.030 mmol) was swollen in 2.0 mL of CH₂Cl₂ for 1 h at room temperature. The tripeptide H₂NAsp(OPMB)LeuThr(OBn)OAll (48.9 mg, 0.0818 mmol) and IIDQ (Aldrich, 24 mL, 0.080 mmol) were successively added. After 16 h, the solvent was filtered and the resin was washed with CH₂Cl₂ (6 X 5 mL) and the resin was dried under reduced pressure to give 117.1 mg of resin 38C.

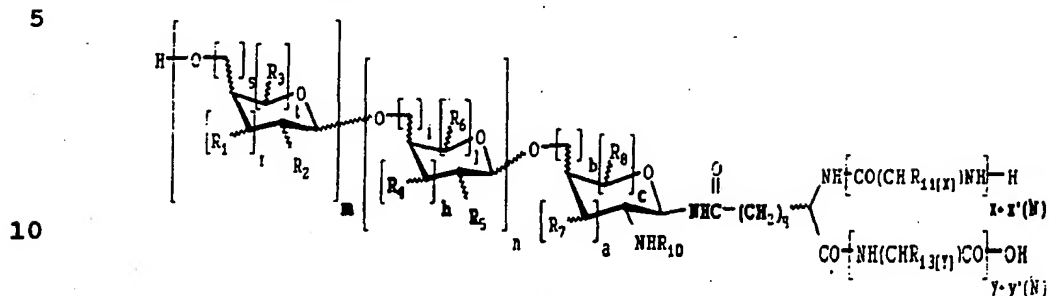
-128-

In a Teflon[™] flask, the polymer-bound trisaccharide-octapeptide 38C (112.9 mg, ~0.0303 mmol) and anisole (8.5 mL, 0.78 mmol) were suspended in 5 mL of CH₂Cl₂ for 1 h at room temperature. The mixture was cooled to -10°C (acetone/ice) and HF·pyridine (~60 mL, 2.0 mmol) was slowly added. After 2 h, H₂O (5 mL) was added at -10°C and the mixture was extracted with ethyl acetate (4 X 30 mL). The combined organic layers were washed twice with a mixture of brine (10 mL) and saturated NaHCO₃ (1-2 mL, pH 7). The organic extracts were dried over sodium sulfate, filtered over a medium porosity glass filter and concentrated. The residue was purified over RP-18 silica [1.5 g, 7:3 MeOH:H₂O (0.1 % TFA) → 9:1] to give trisaccharide octapeptide 39C (11.3 mg, ~18%): [α]_D²⁴ -6.6° (c 0.66, CH₂Cl₂); HRMS (FAB) calcd for C₁₀₀¹³CH₁₂₉N₁₀O₃₅Na₂ 2088.8417, found 2088.8425.

-129-

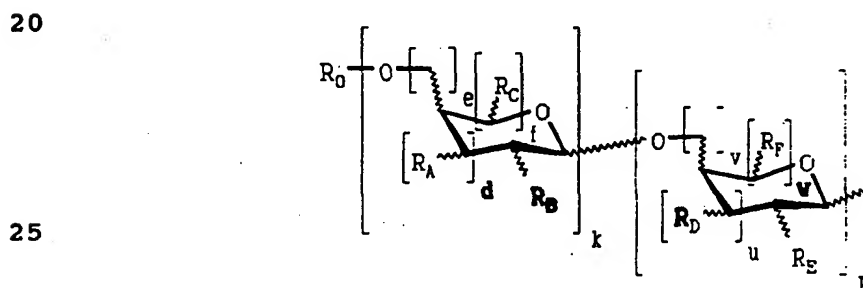
What is claimed is:

1. A process of synthesizing a glycop ptide having the structure:



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wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are each independently H, OH, OR^i , NH_2 , $NHCOR^i$, F, CH_2OH , CH_2OR^i , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R^i is H, CHO, CO_2R^{ii} , a linear or branched chain alkyl, arylalkyl or aryl group, or an oligosaccharide moiety having the structure:



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wherein R_0 is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein d, e, f, k, p, u, v and w are each independently 0, 1 or 2; wherein R_A , R_B , R_C , R_D , R_E and R_F are each independently H, OH, OR^{iii} , NH_2 , $NHCOR^{iii}$, F, CH_2OH , CH_2OR^{iii} , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, wherein R^{iii} is H, CHO, CO_2R^{iv} , a linear or branched chain alkyl, arylalkyl or aryl group, and wherein R^{ii} and R^{iv} are independently a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group;

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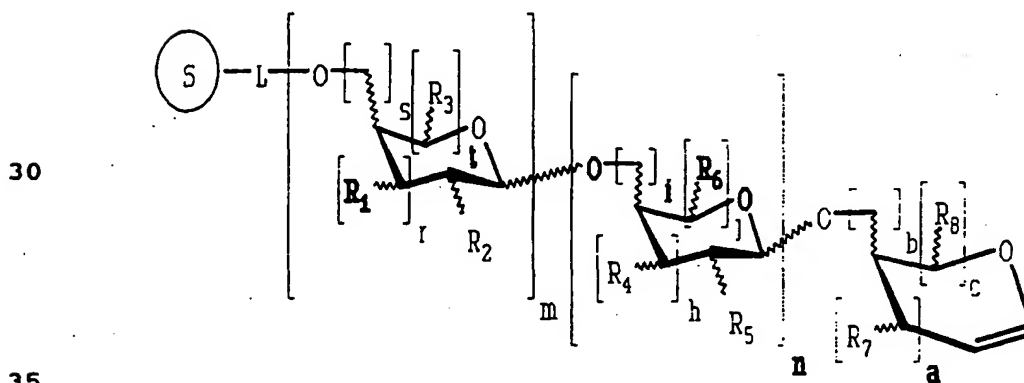
wherein R_{10} is a substituted or unsubstituted linear

-130-

or branched chain acyl, arylacyl or aroyl group;
 wherein $R_{11}[X]$ represent X amino acid side-chains,
 where X is an integer from 1 to $x+x'(N)$, and denotes
 position from the N-terminus, and $x'(N)$ is a
 5 summation over N , where N is an integer from 1 to
 10, and $R_{13}[Y]$ represent Y amino acid side-chains,
 where Y is an integer from 1 to $y+y'(N)$, and denotes
 position from the C-terminus, and $y'(N)$ is a
 10 summation over N , where N is an integer from 1 to
 10, wherein each $R_{11}[X]$ and $R_{13}[Y]$ are independently
 the same or different, and are H, OH, a substituted
 or unsubstituted linear or branched chain alkyl,
 arylalkyl or aryl group; wherein $a, b, c, h, i, j,$
 15 r, s and t are each independently an integer between
 about 0 and about 3; wherein m and n are each
 independently an integer between about 0 and about
 5; wherein q is an integer between about 1 and about
 9; and wherein $x, x'(N), y$ and $y'(N)$ are each
 20 independently an integer between about 0 and about
 25;

which comprises:

(a) halosulfonamidating a compound having the
 25 structure:



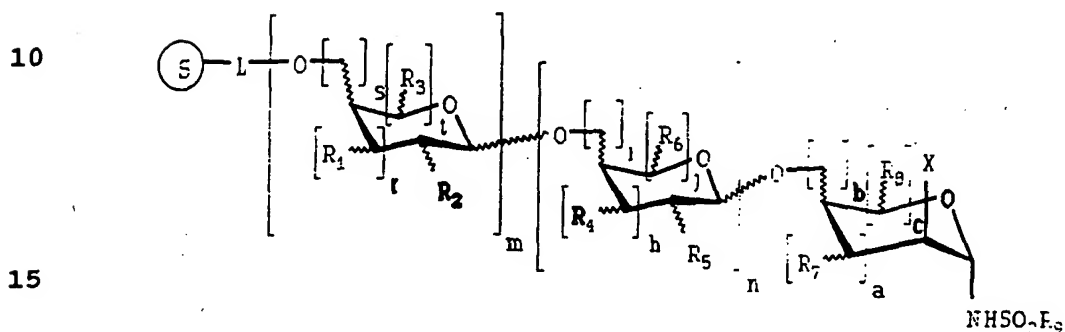
wherein L is a suitable linking moiety
 selected from the group consisting of SiR_2 , a
 40 substituted or unsubstituted linear or branched
 chain alkyl, arylalkyl, and aryl groups, where

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R is a linear or branched chain alkyl, alkoxy, arylalkyl, arylalkoxy or aryl group;

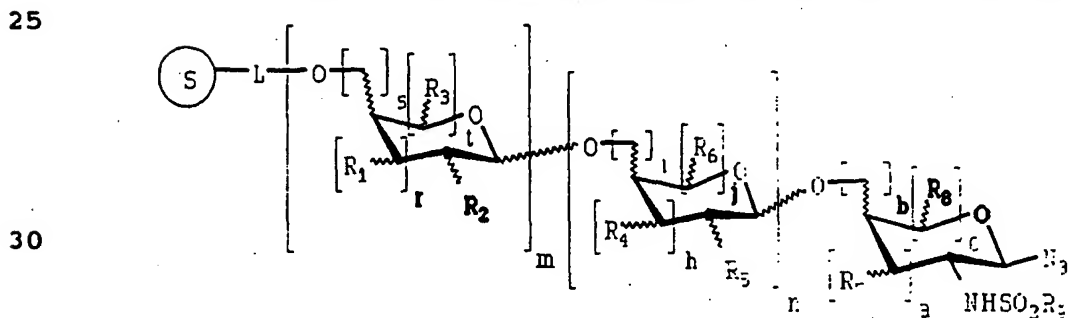
wherein (s) is a polymeric solid-phase; with a

compound having the formula $R_9SO_2NH_2$, wherein R_9 is a substituted or unsubstituted, or a linear or branched chain alkyl, arylalkyl or aryl group under suitable conditions to form a compound having the structure:



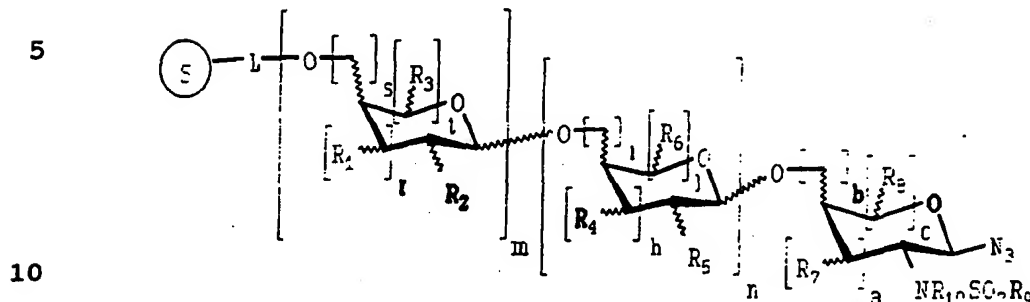
wherein X is selected from the group consisting of F, Cl, Br and I;

(b) reacting the compound formed in step (a) with an azide salt under suitable conditions to form a sulfonamide azide having the structure:

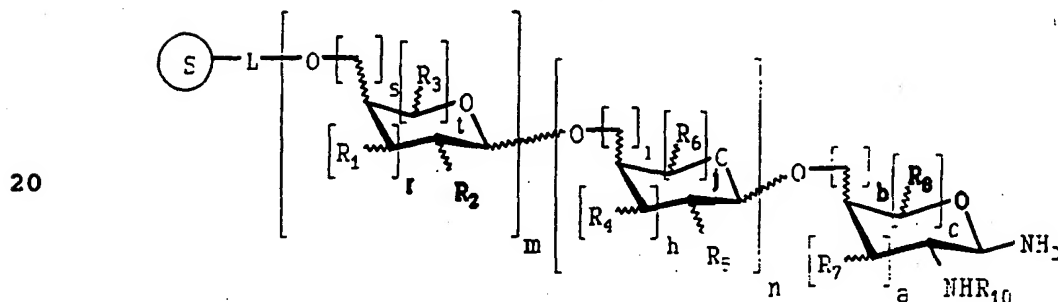


-132-

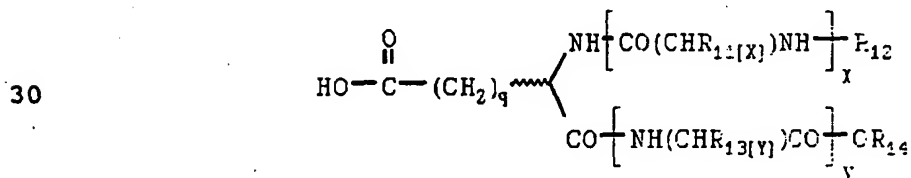
- (c) acylating the sulfonamide azide formed in step (b) under suitable conditions to form an N-acylsulfonamide having the structure:



- (d) reducing the N-acylsulfonamide formed in step (c) with a reducing agent under suitable conditions to form an amine N-acylamide having the structure:



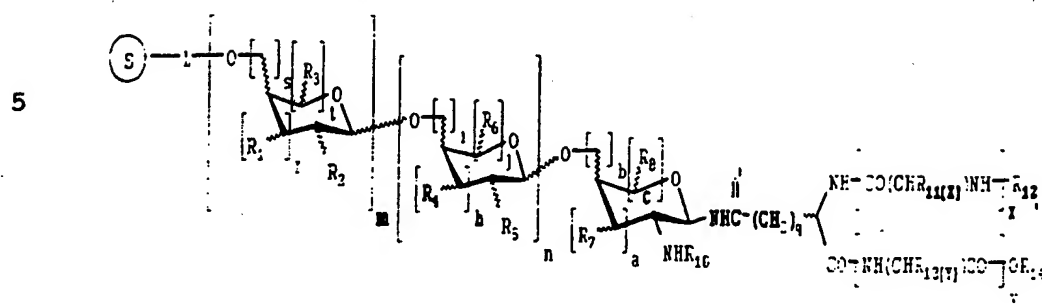
- (e) coupling the amine N-acylamide with a suitably protected acidic peptide having the structure:



wherein R_{12} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; wherein R_{14} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein X is an integer between about 1 and x; under suitable conditions to

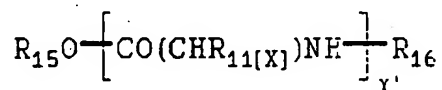
-133-

form a protected glycopeptide having the structure:

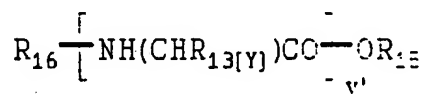


(f) (i) selectively deprotecting the protected glycopeptide formed in step (e) under suitable conditions to form either an N- or C-deprotected glycopeptide;

(ii) coupling the N- or C-deprotected glycopeptide respectively under suitable conditions with a protected amino acid or oligopeptide having the structure:

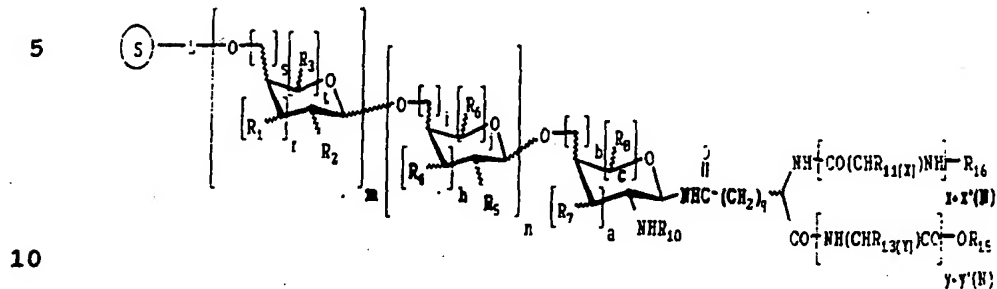


wherein R_{15} is H; and wherein R_{16} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; or having the structure:



wherein R_{16} is H; and wherein R_{15} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and

(iii) optionally repeating iteratively steps (i) and (ii) N times to form a chain-extended glycopptide having the structure:



and

(g) cleaving and deprotecting the chain-extended glycopeptide under suitable conditions to form the glycopeptide.

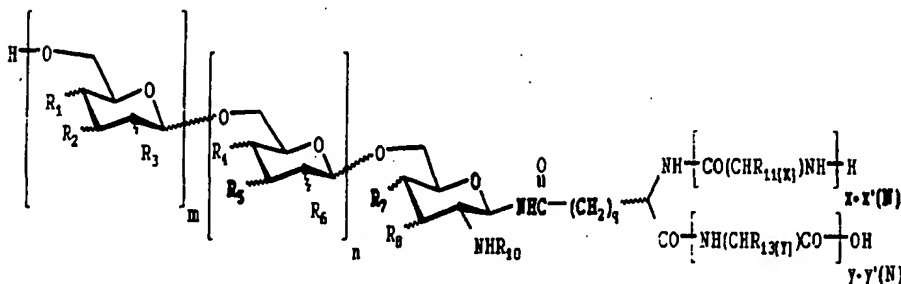
2. The process of claim 1 wherein b, i and s are each 1.
3. The process of claim 1 wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are OH.
4. The process of claim 1 wherein X is I and R_9 is 10-anthracenyl.
5. The process of claim 1 wherein R is isopropyl.
6. The process of claim 1 wherein the polymeric solid phase is selected from the group consisting of a polystyrene resin, silica gel, glass beads, an agarose resin and a polyacrylamide resin.
7. The process of claim 1 wherein the polymeric solid phase is cross-linked polystyrene.
8. The process of claim 7 wherein the solid phase is cross-linked with 1% divinyl benzene.
9. The process of claim 1 wherein a, c, h, j, r, t, m and n are each 1.

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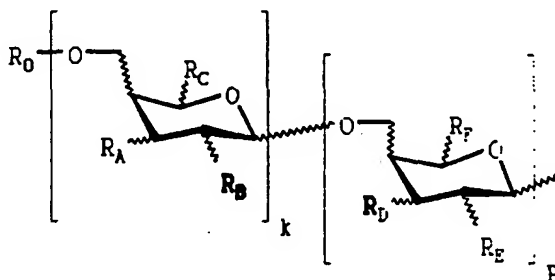
10. The process of claim 1 wherein R_{12} is selected from the group consisting of t-butyloxycarbonyl, t-amylloxycarbonyl, 9-fluorenylmethyloxycarbonyl, benzyloxycarbonyl and biphenylisopropylloxycarbonyl.

11. The process of claim 1 wherein R_{14} is selected from the group consisting of methyl, ethyl, t-butyl, benzyl, p-bromobenzyl, 2,4-dichlorobenzyl, α,α -dimethylbenzyl, trityl, phenacyl and benzhydryl.

12. A process of synthesizing a glycopeptide having the structure:



wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are each independently H, OH, OR^i , NH_2 , $NHCOR^i$, F, CH_2OH , CH_2OR^i , or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, where R^i is H, CHO, CO_2R^{ii} , a linear or branched chain alkyl, arylalkyl or aryl group, or an oligosaccharide moiety having the structure:



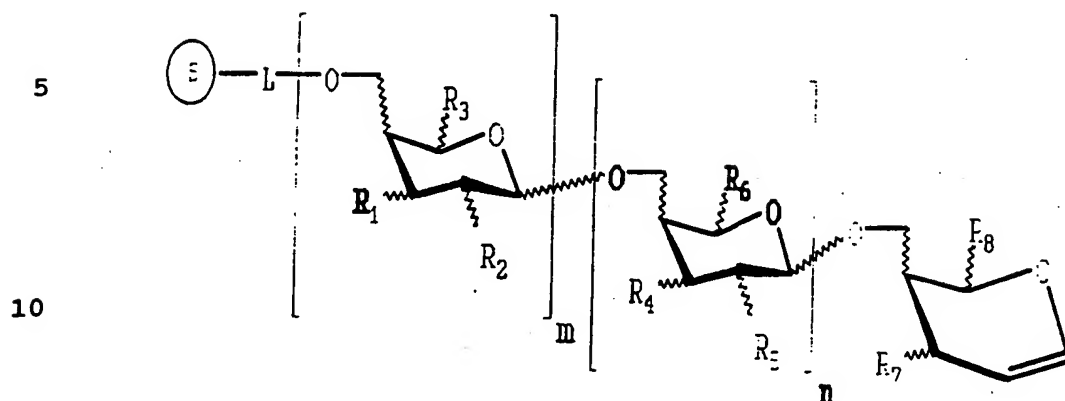
wherein R_0 is H, a linear or branched chain alkyl, arylalkyl or aryl group; wherein k and p, are each independently 0, 1 or 2; wherein R_A , R_B , R_C , R_D , R_E and R_F are each independently H, OH, OR^{iii} , NH_2 ,

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$\text{NHCOR}^{\text{iii}}$, F, CH_2OH , $\text{CH}_2\text{OR}^{\text{iii}}$, or a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group, wherein R^{iii} is H, CHO, $\text{CO}_2\text{R}^{\text{iv}}$, a linear or branched chain alkyl, arylalkyl or aryl group, and wherein R^{ii} and R^{iv} are independently a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein R_{10} is a substituted or unsubstituted linear or branched chain acyl, arylacyl or aroyl group; wherein $\text{R}_{11[\text{x}]}$ represent X amino acid side-chains, where X is an integer from 1 to $\text{x}+\text{x}'(\text{N})$, and denotes position from the N-terminus, and $\text{x}'(\text{N})$ is a summation over N, where N is an integer from 1 to 10, and $\text{R}_{13[\text{y}]}$ represent Y amino acid side-chains, where Y is an integer from 1 to $\text{y}+\text{y}'(\text{N})$, and denotes position from the C-terminus, and $\text{y}'(\text{N})$ is a summation over N, where N is an integer from 1 to 10, wherein each $\text{R}_{11[\text{x}]}$ and $\text{R}_{13[\text{y}]}$ are independently the same or different, and are H, OH, a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein m and n are each independently an integer between about 0 and about 5; wherein q is an integer between about 1 and about 9; and wherein x, $\text{x}'(\text{N})$, y and $\text{y}'(\text{N})$ are each independently an integer between about 0 and about 25;

which comprises:

(a) halosulfonamidating a compound having the structure:

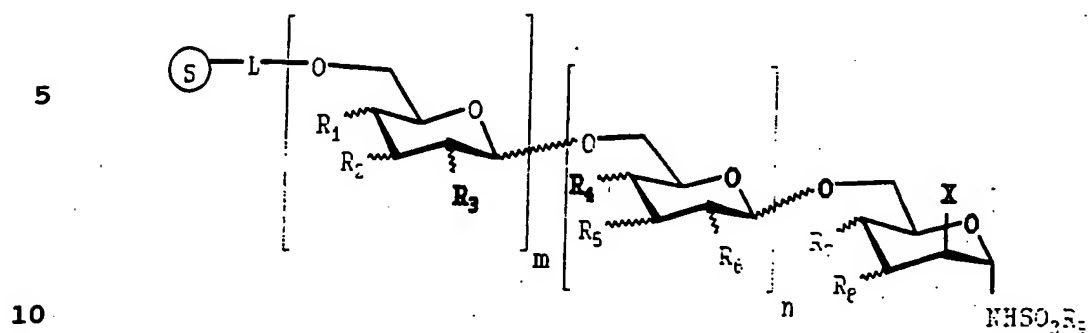


wherein L is a suitable linking moiety selected from the group consisting of SiR_2 , a substituted or unsubstituted linear or branched chain alkyl, arylalkyl, and aryl groups, where R is a linear or branched chain alkyl, alkoxy, arylalkyl, arylalkoxy or aryl group; wherein (s) is a polymeric solid-phase; with a

compound having the formula $R_9SO_2NH_2$, wherein R_9 is a substituted or unsubstituted, or a linear or branched chain alkyl, arylalkyl or

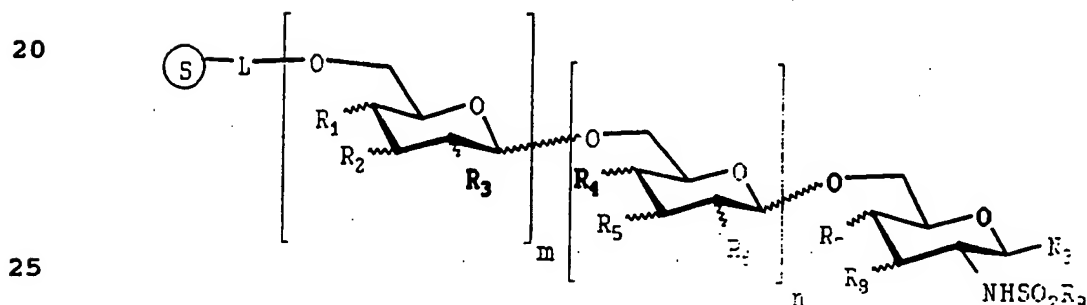
-138-

aryl group under suitable conditions to form a compound having the structure:

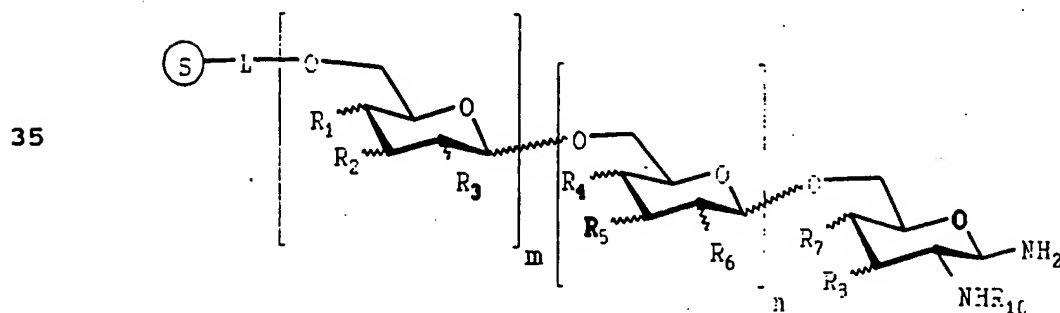


wherein X is selected from the group consisting of F, Cl, Br and I;

(b) reacting the compound formed in step (a) with an azide salt under suitable conditions to form a sulfonamide azide having the structure:

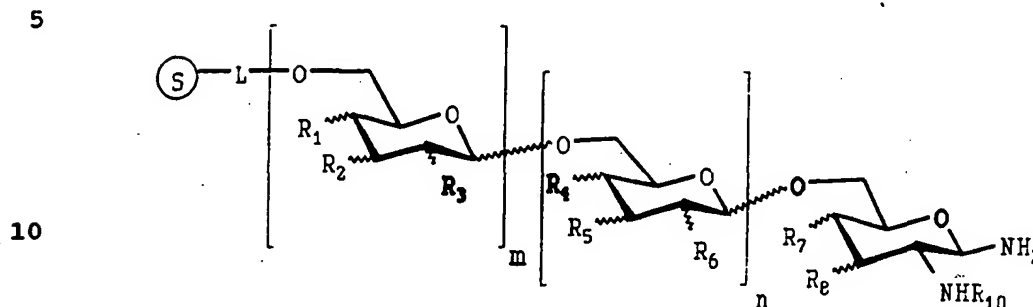


(c) acylating the sulfonamide azide formed in step (b) under suitable conditions to form an N-acylsulfonamide having the structure:

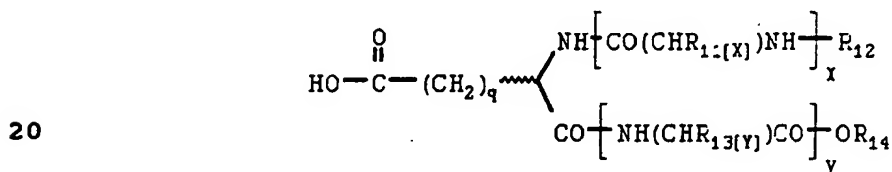


-139-

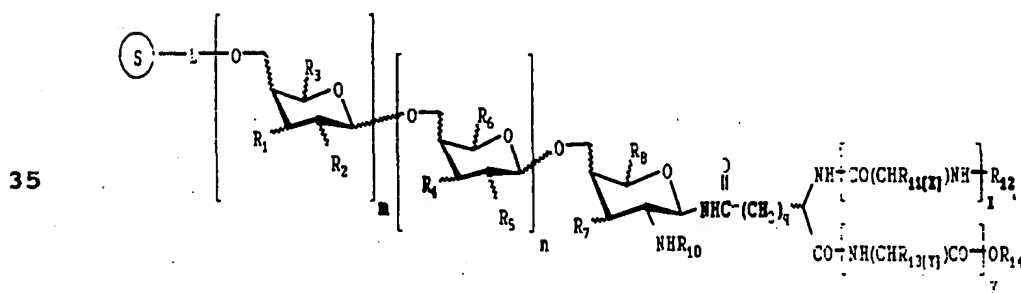
- (d) reducing the N-acysulfonamide formed in step (c) with a reducing agent under suitable conditions to form an amine N-acylamide having the structure:



- (e) coupling the amine N-acylamide with a suitably protected acidic peptide having the structure:



wherein R_{12} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; wherein R_{14} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; wherein X is an integer between about 1 and x ; under suitable conditions to form a protected glycopeptide having the structure:



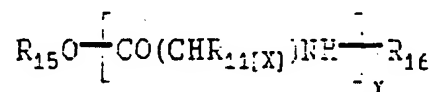
- (f) (i) selectively protecting the protected glycopeptide from (d) in step (e) under suitable

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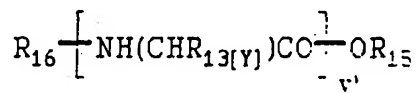
-140-

conditions to form either an N- or C-deprotected glycopeptide;

(ii) coupling the N- or C-deprotected glycopeptide respectively under suitable conditions with a protected amino acid or oligopeptide having the structure:

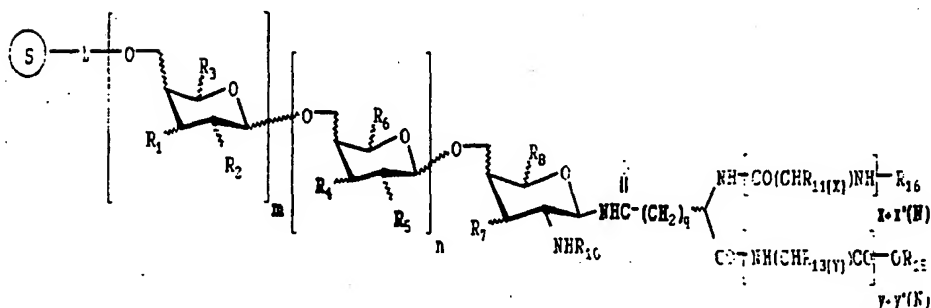


wherein R_{15} is H; and wherein R_{16} is a substituted or unsubstituted alkyl or aryl carbamate or a linear or branched alkyl, acyl, arylacyl, aryl group; or having the structure:



wherein R_{16} is H; and wherein R_{15} is a substituted or unsubstituted linear or branched chain alkyl, arylalkyl or aryl group; and

(iii) optionally repeating iteratively steps (i) and (ii) N times to form a chain-extended glycopeptide having the structure:



and

(g) cleaving and deprotecting the chain-extended glycopeptide under suitable conditions to form the glycopeptide.

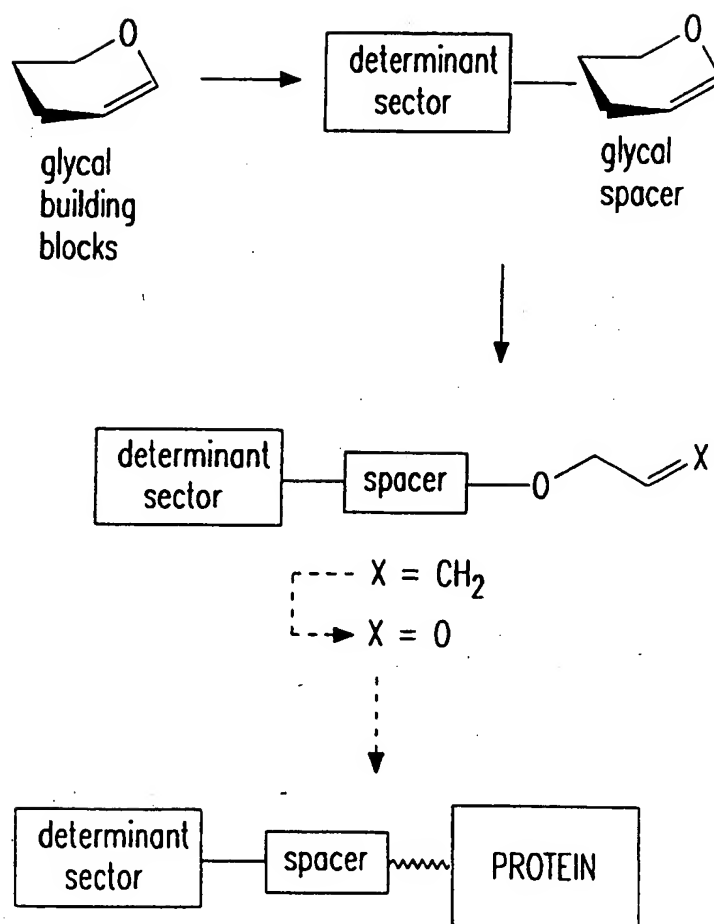
13. The process of claim 12 wherein R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 and R_8 are OH.

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14. The process of claim 12 wherein wherein X is I and R₉ is 10-anthracenyl.
15. The process of claim 12 wherein R is isopropyl.
16. The process of claim 12 wherein the polymeric solid phase is cross-linked polystyrene.
17. The process of claim 16 wherein the solid phase is cross-linked with 1% divinyl benzene.
18. The process of claim 12 wherein m and n are each 1.
19. The process of claim 12 wherein R₁₂ is selected from the group consisting of t-butyloxycarbonyl, t-amyl-oxy carbonyl, 9-fluorenylmethyloxycarbonyl, benzyl-oxy carbonyl, and biphenylisopropyloxycarbonyl.
20. The process of claim 12 wherein R₁₄ is selected from the group consisting of methyl, ethyl, t-butyl, benzyl, p-bromobenzyl, 2,4-dichlorobenzyl, α,α -dimethylbenzyl, trityl, phenacyl, and benzhydryl.

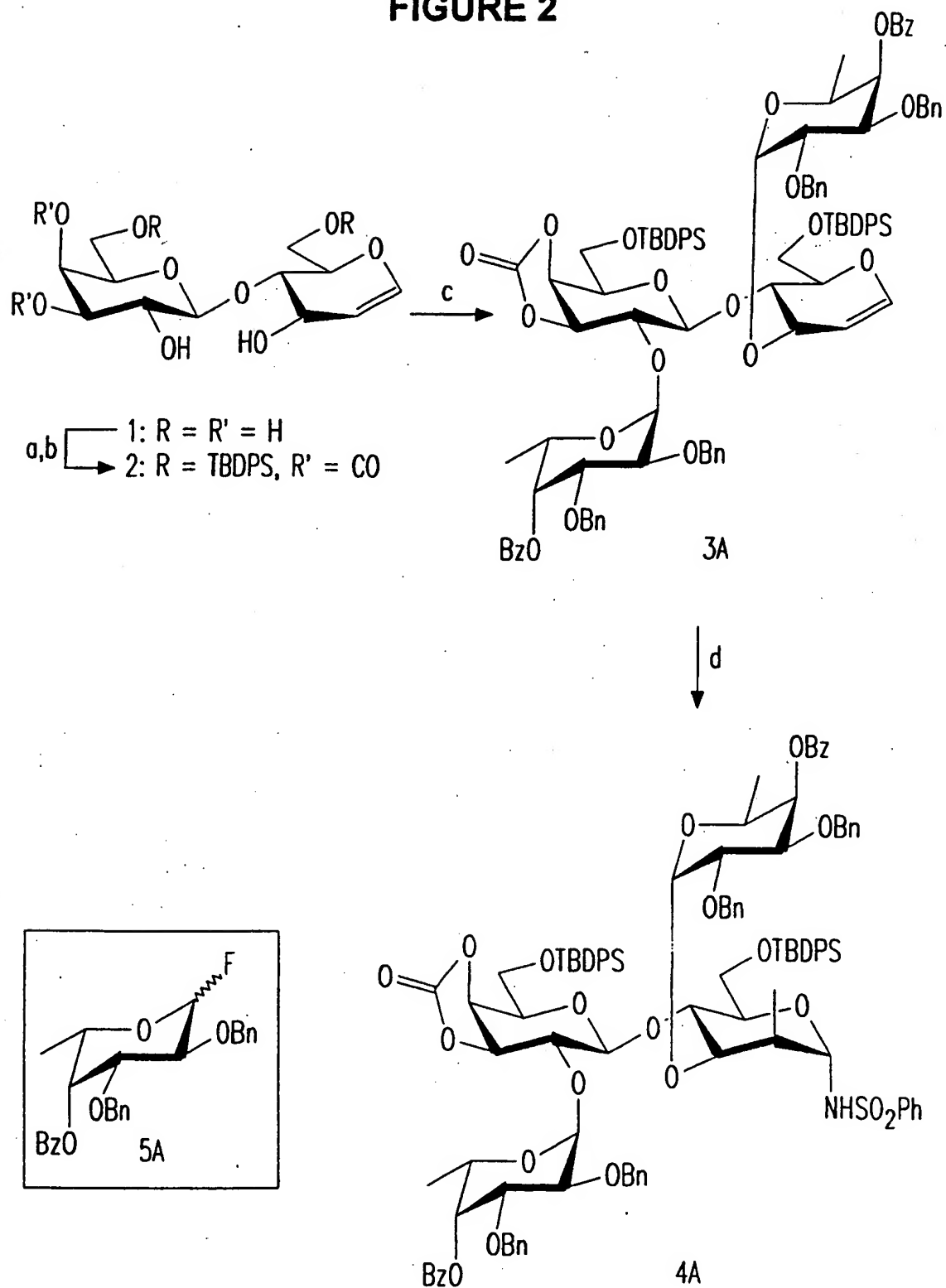
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FIGURE 1



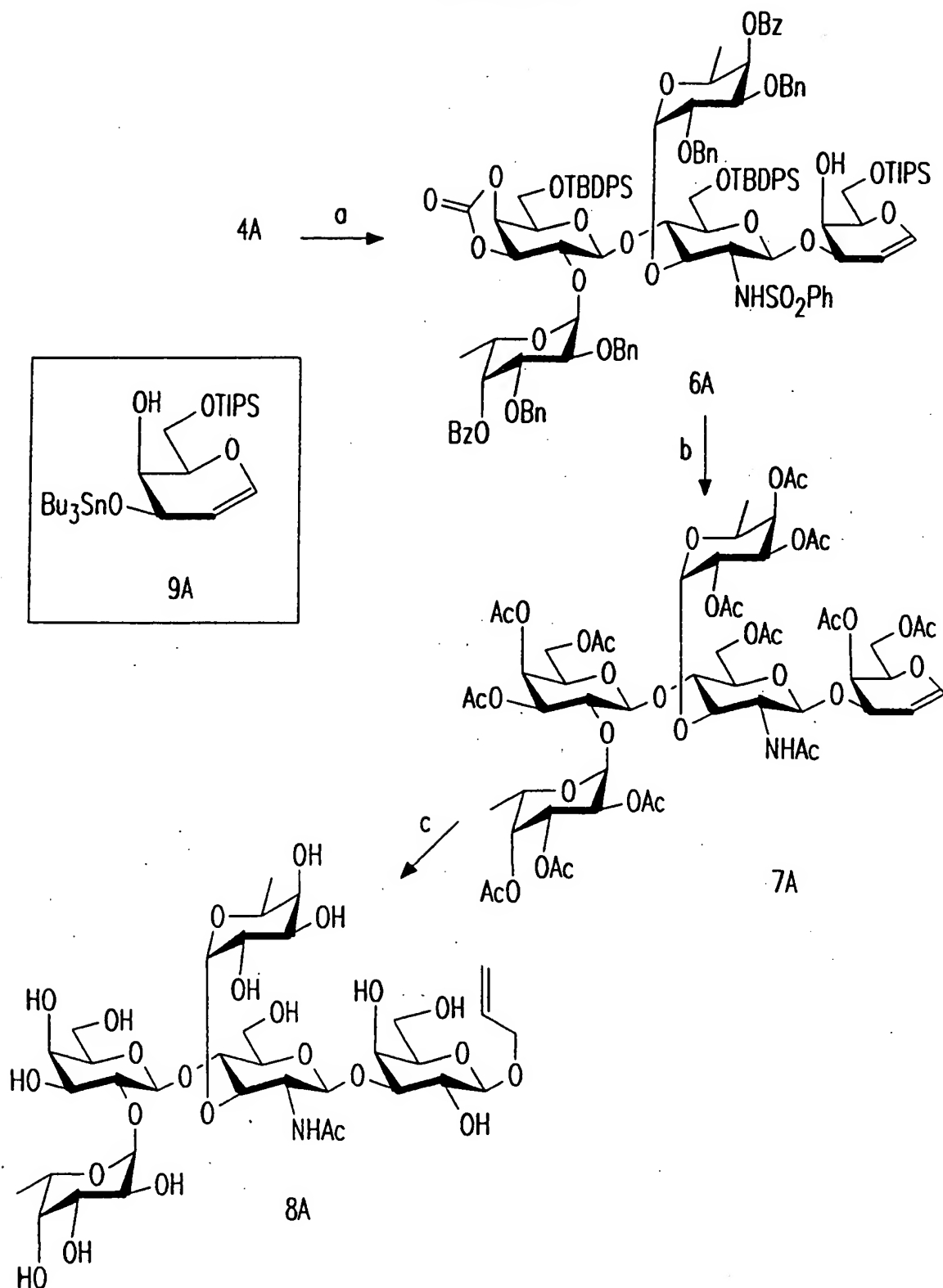
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FIGURE 2

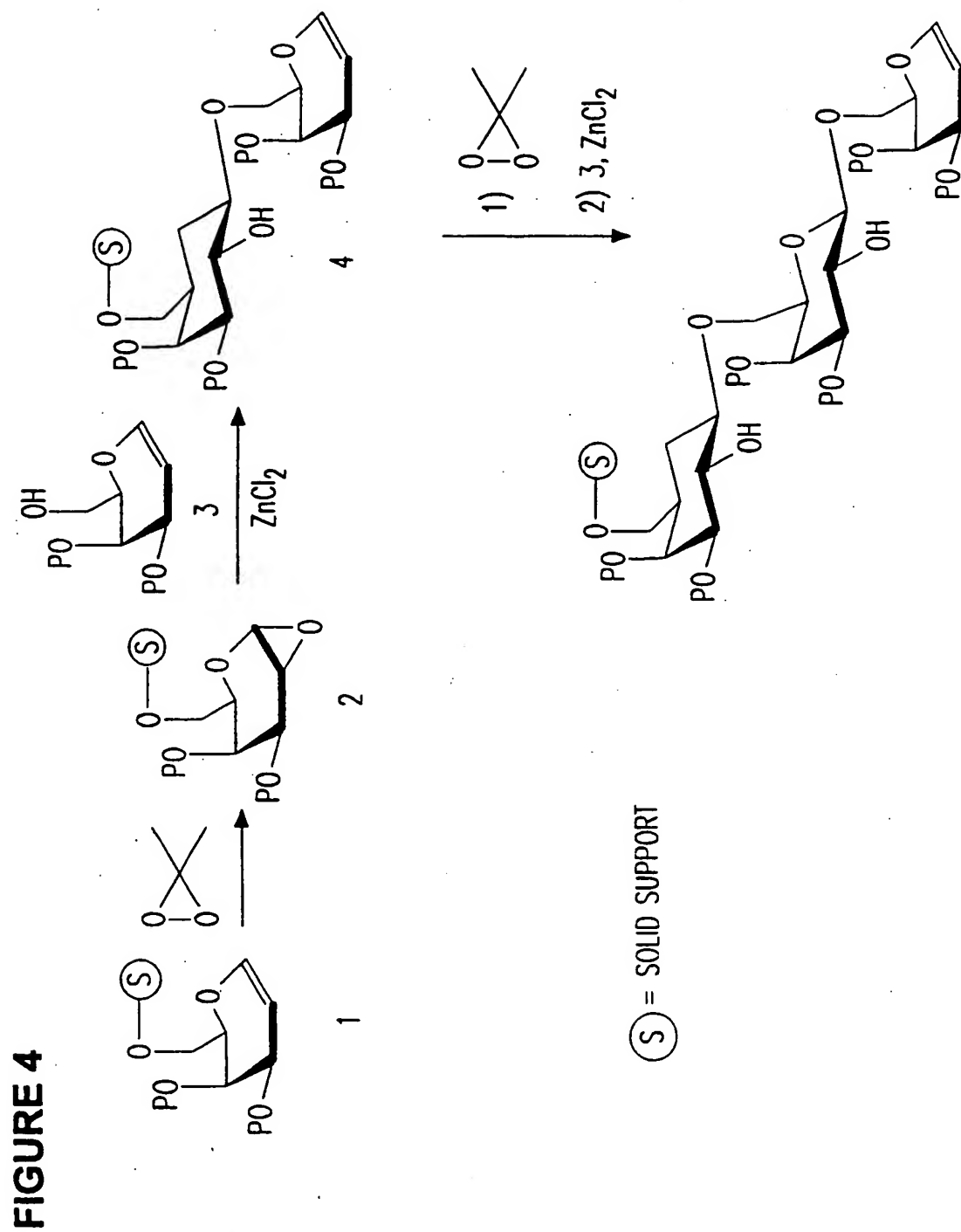


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FIGURE 3

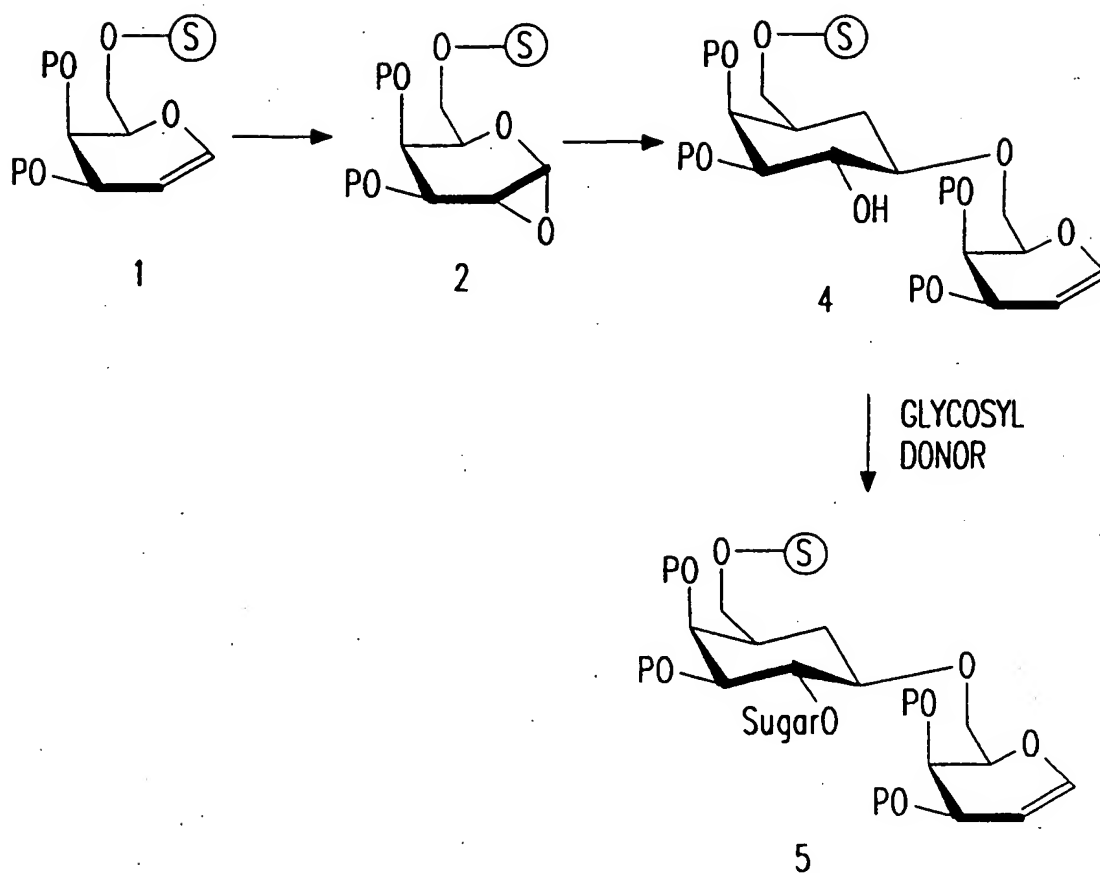


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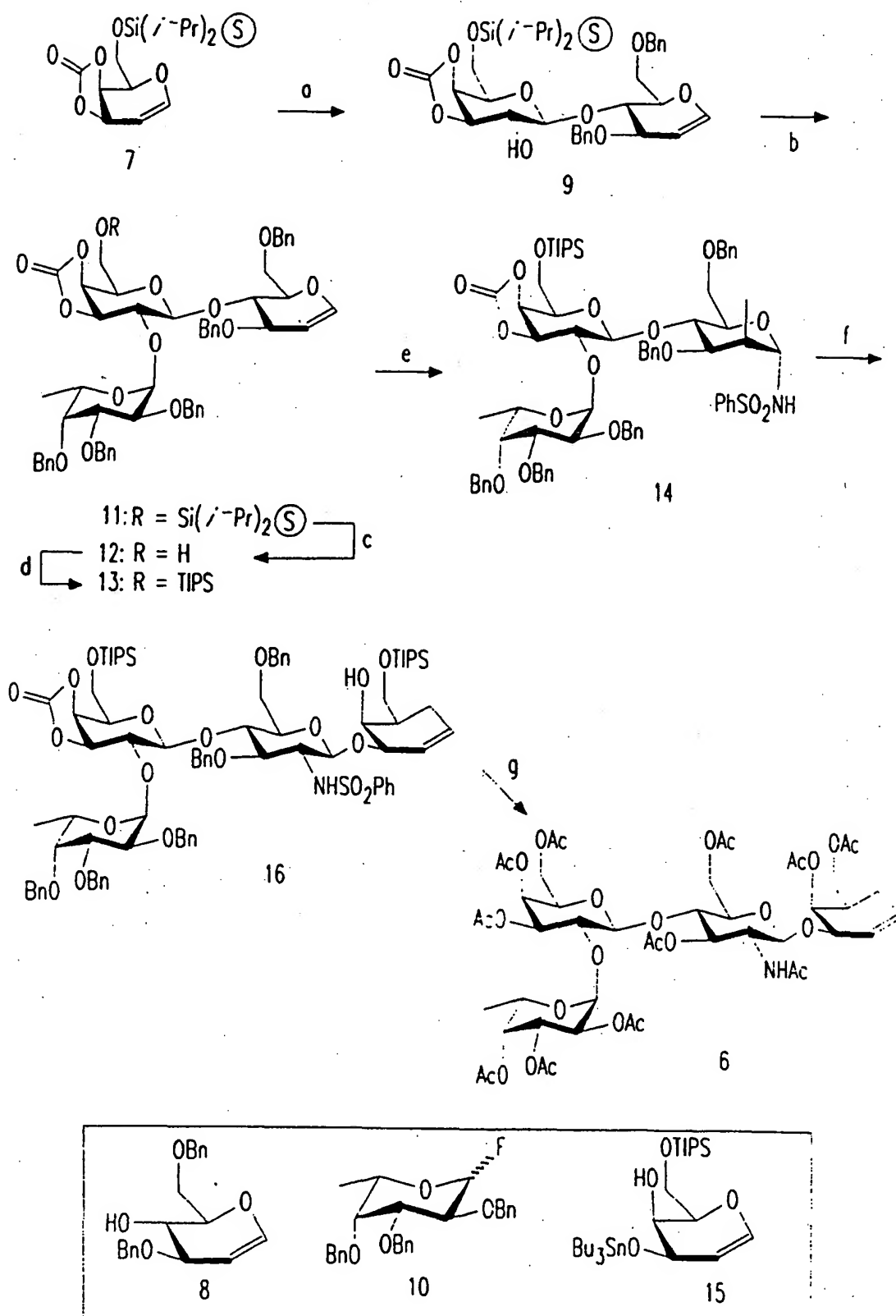
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FIGURE 5



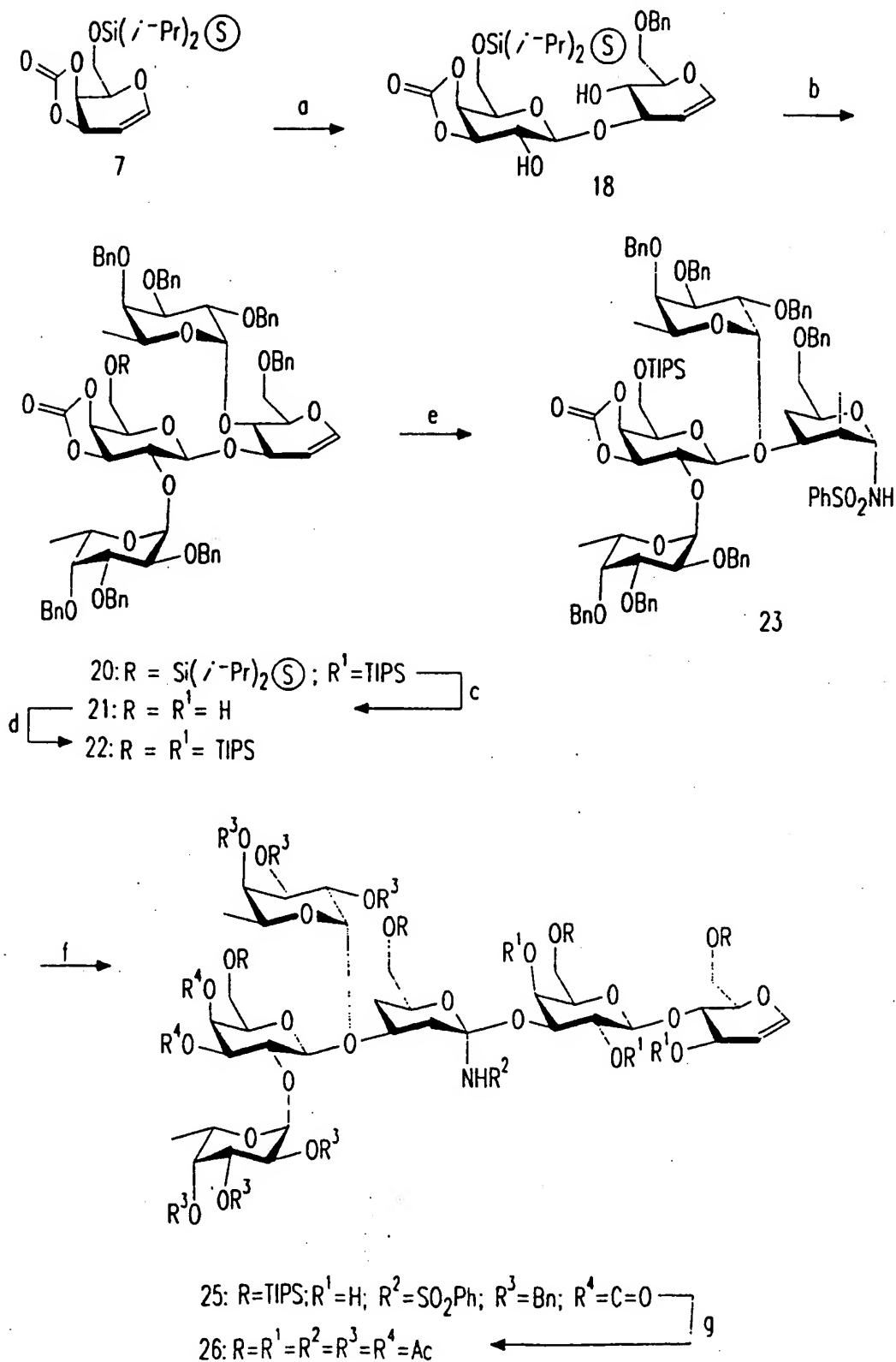
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FIGURE 6



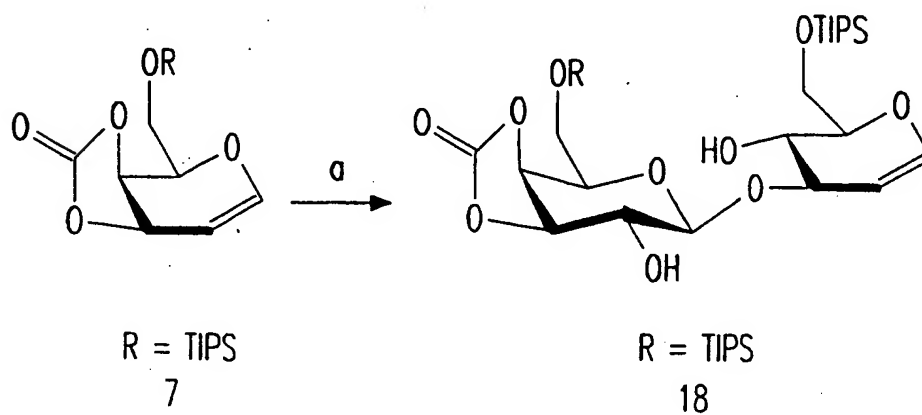
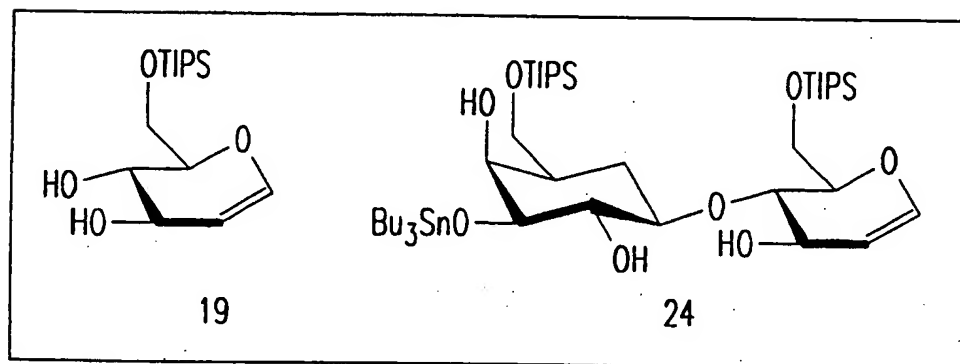
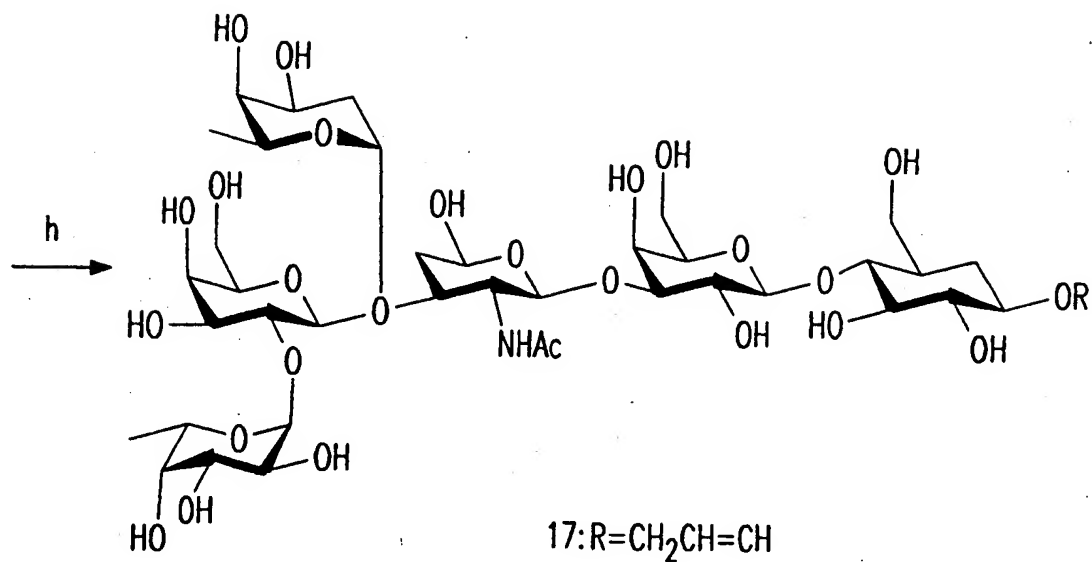
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FIGURE 7A



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FIGURE 7b



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FIGURE 8A

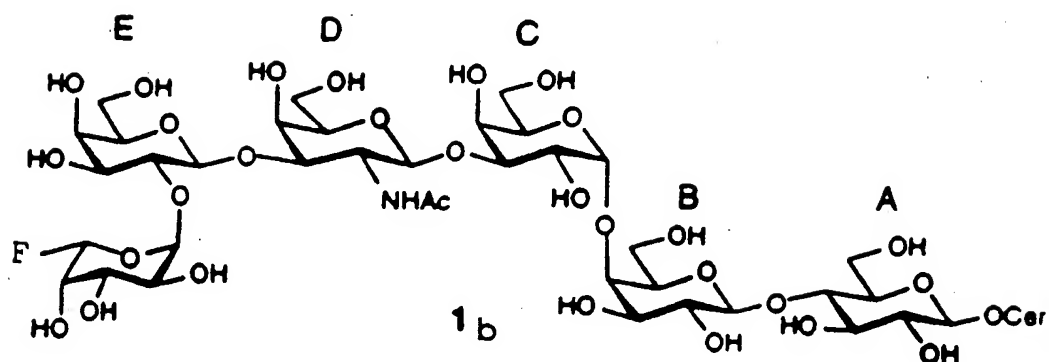
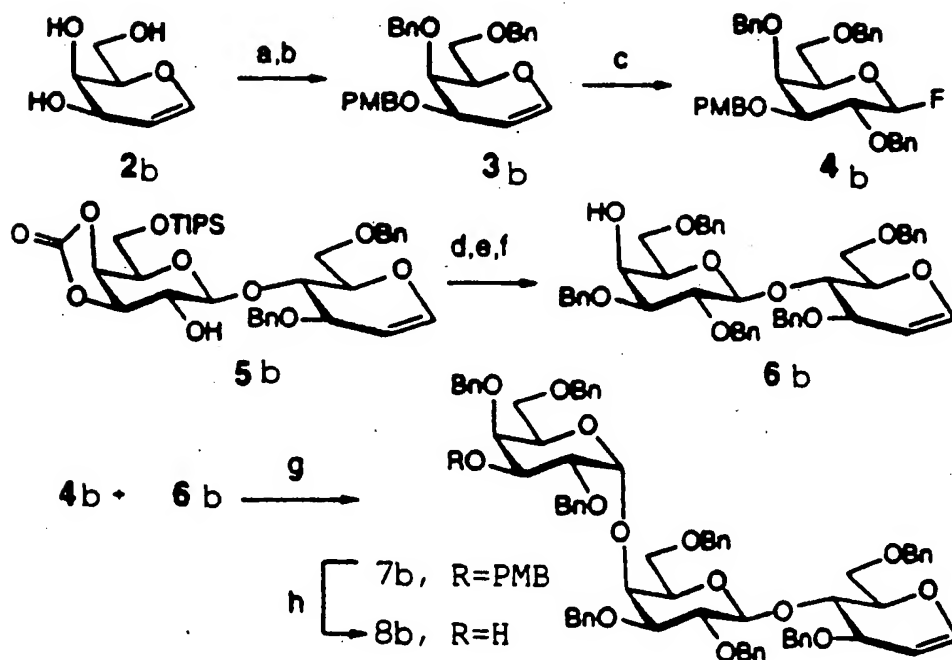
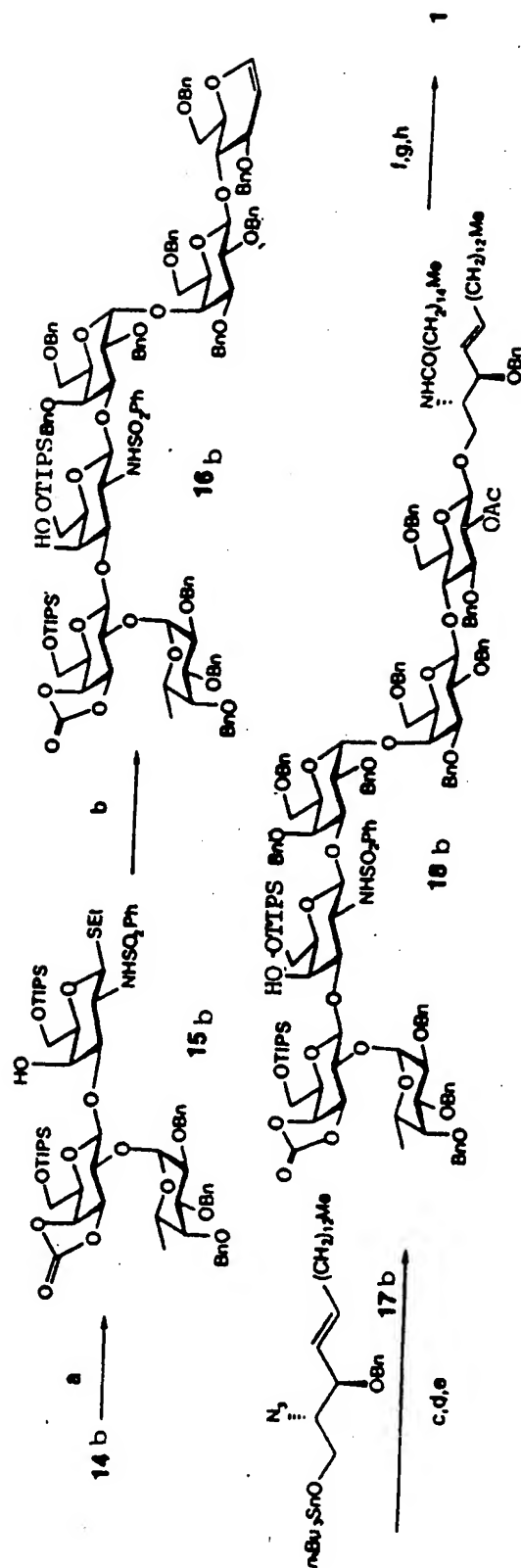


FIGURE 8B



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FIGURE 9



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FIGURE 10A

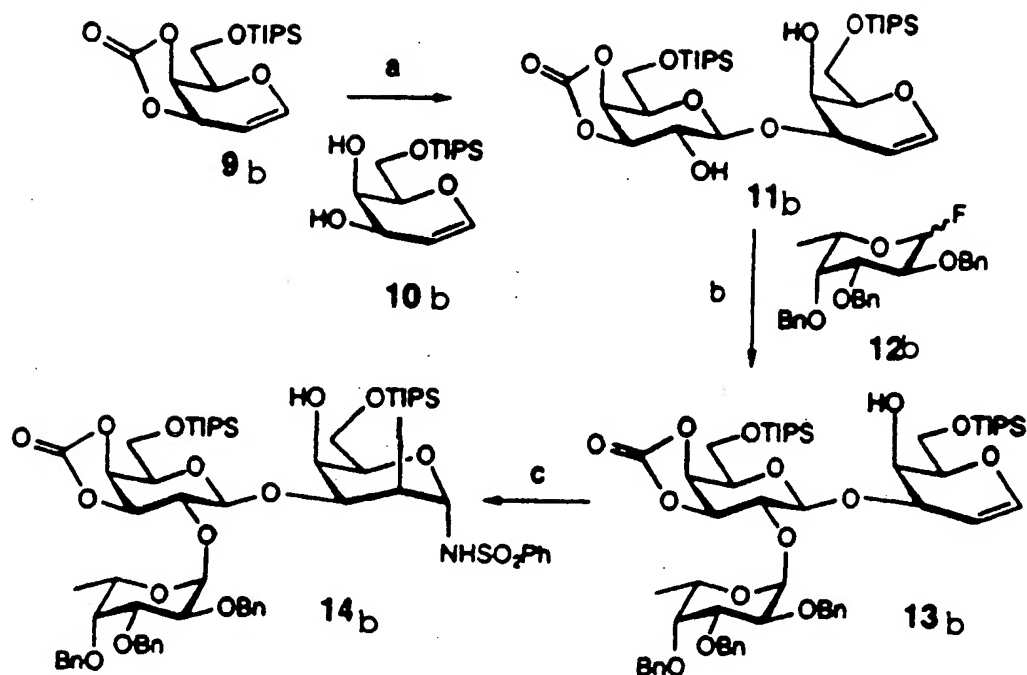
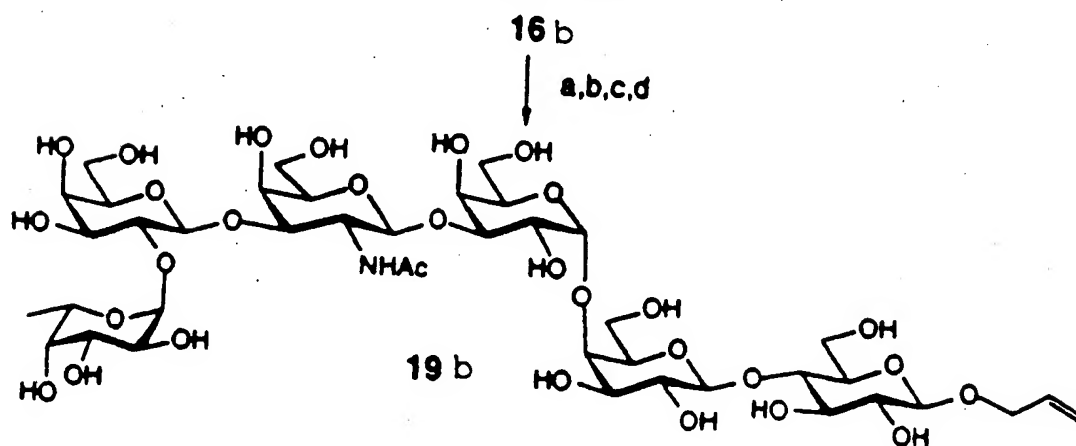
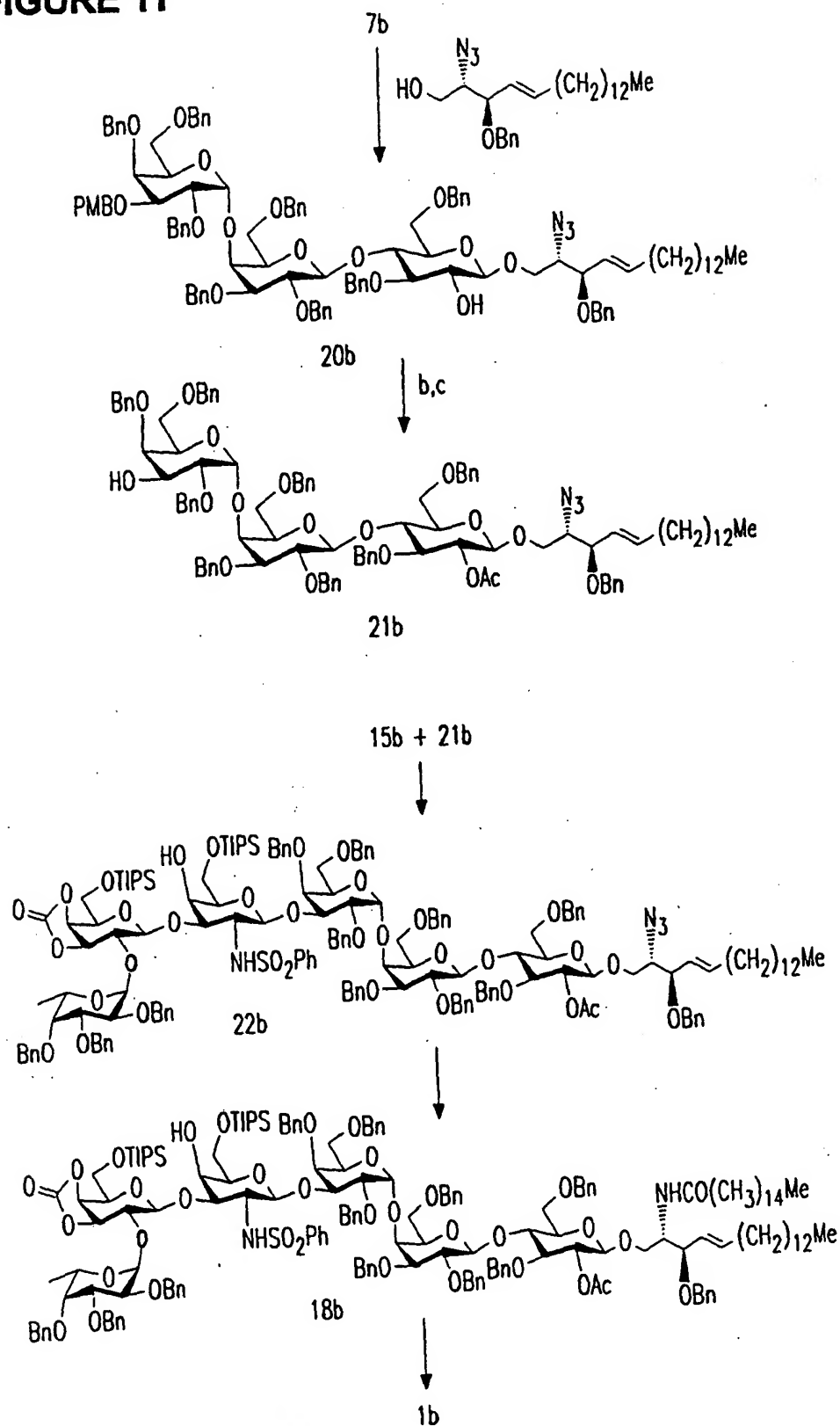


FIGURE 10B



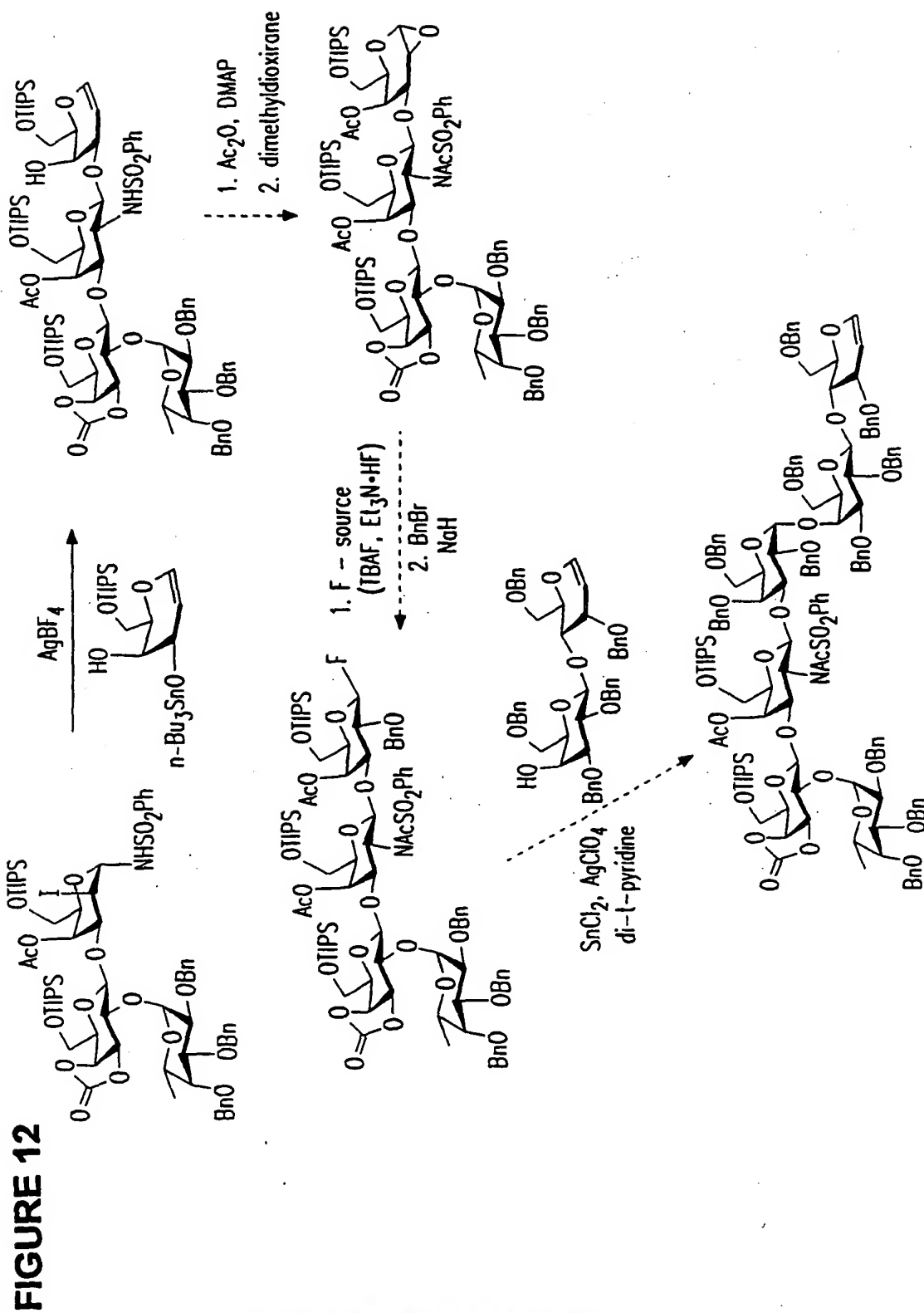
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FIGURE 11



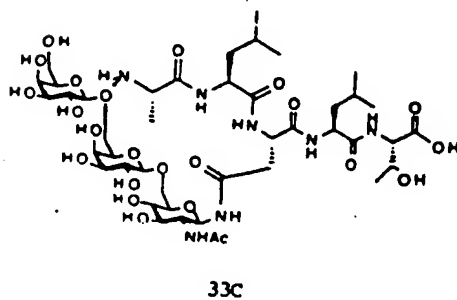
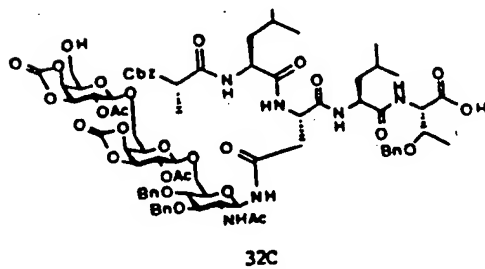
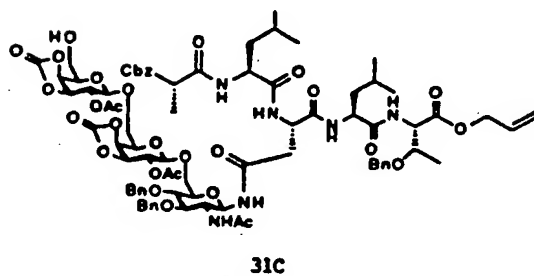
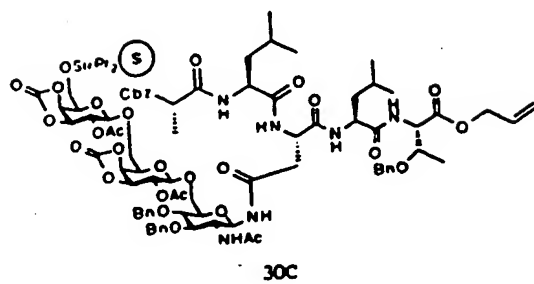
SUBSTITUTE SHEET (RHF 26)

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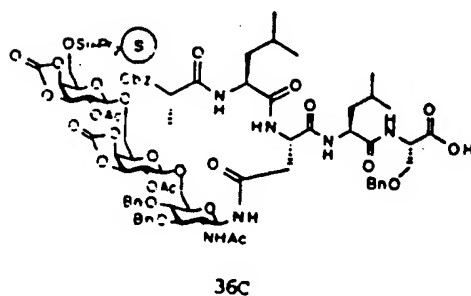
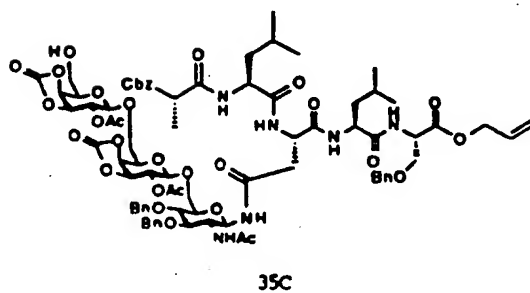
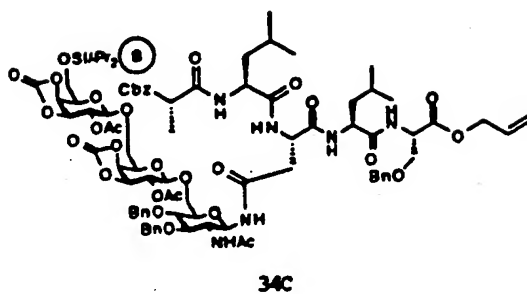
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FIGURE 13A



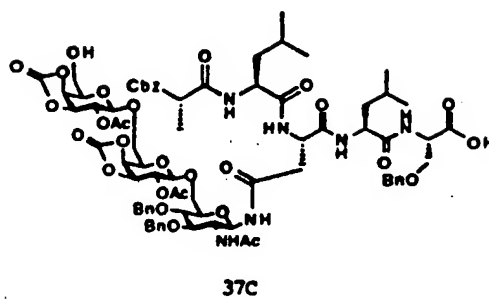
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FIGURE 13B

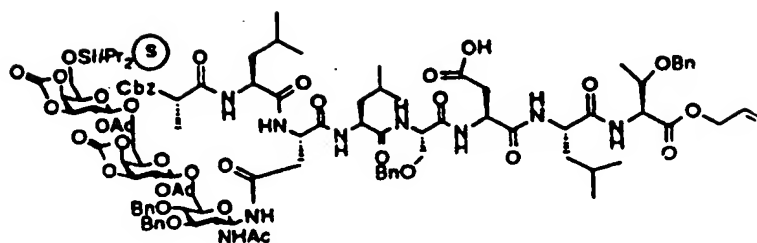


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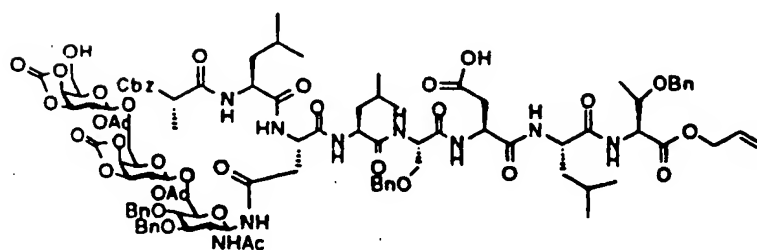
FIGURE 13C



37C



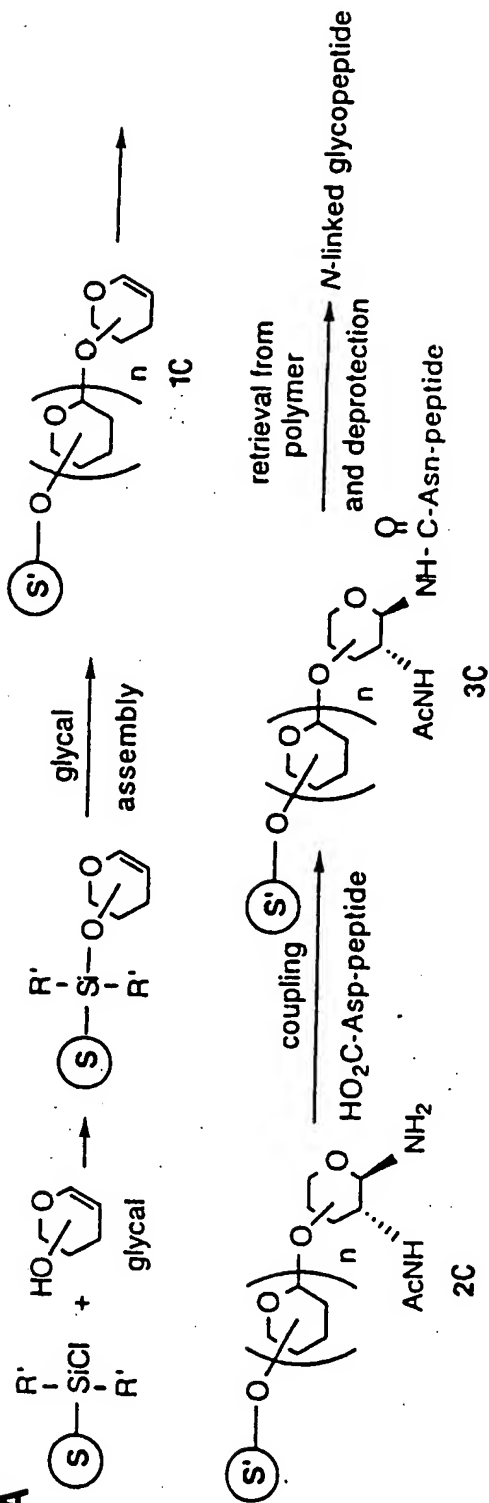
38C



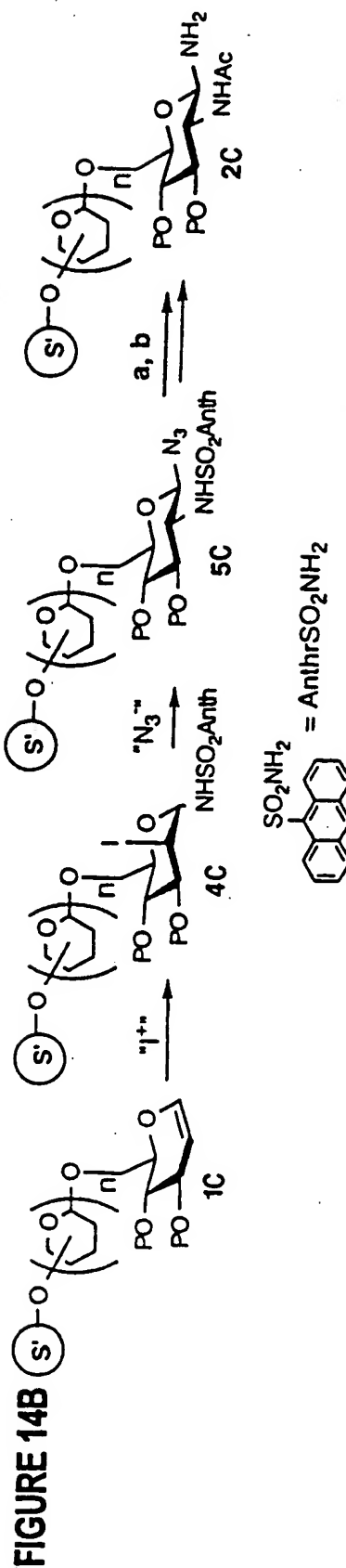
39C

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FIGURE 14A



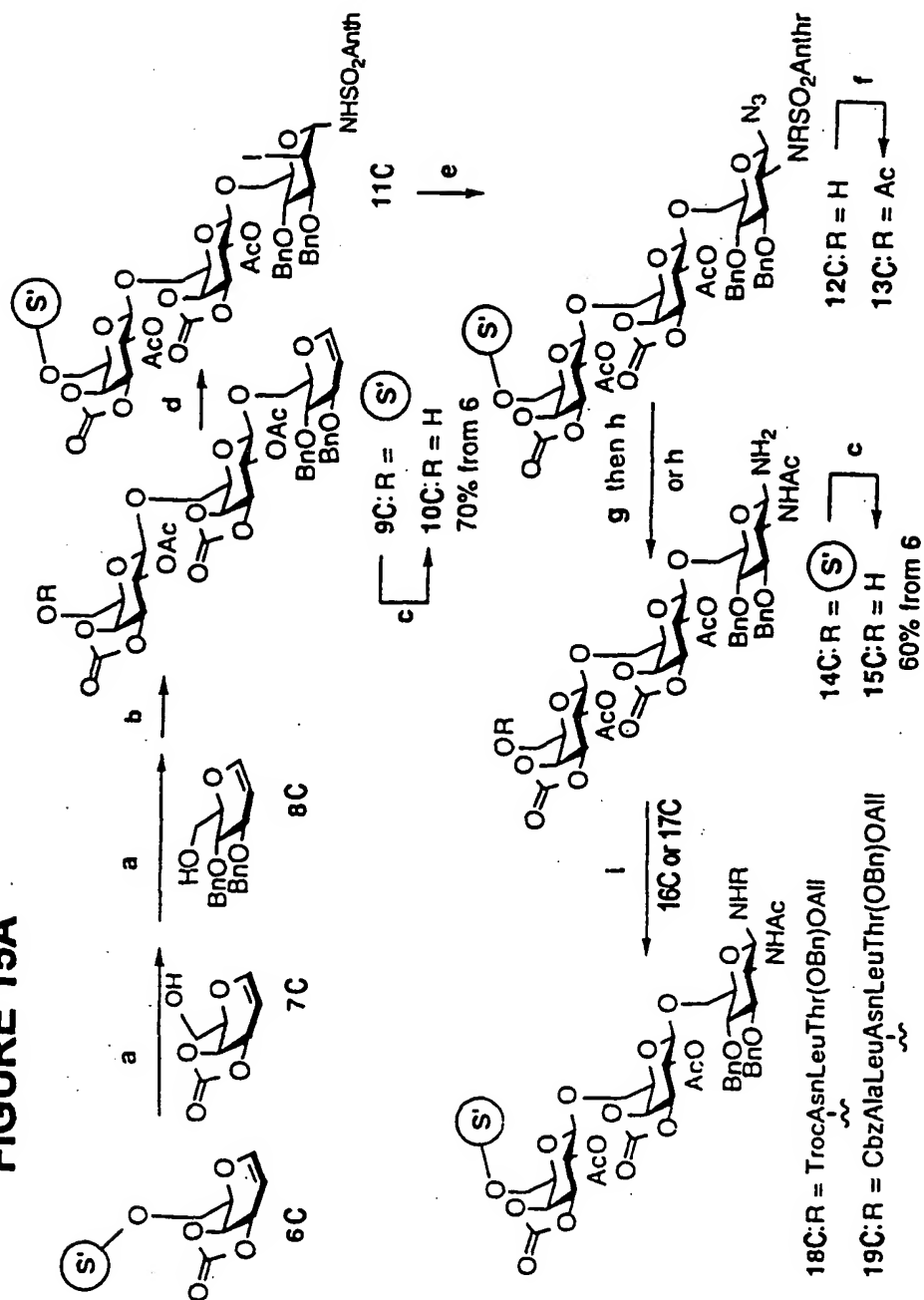
Scheme I:



Scheme II:

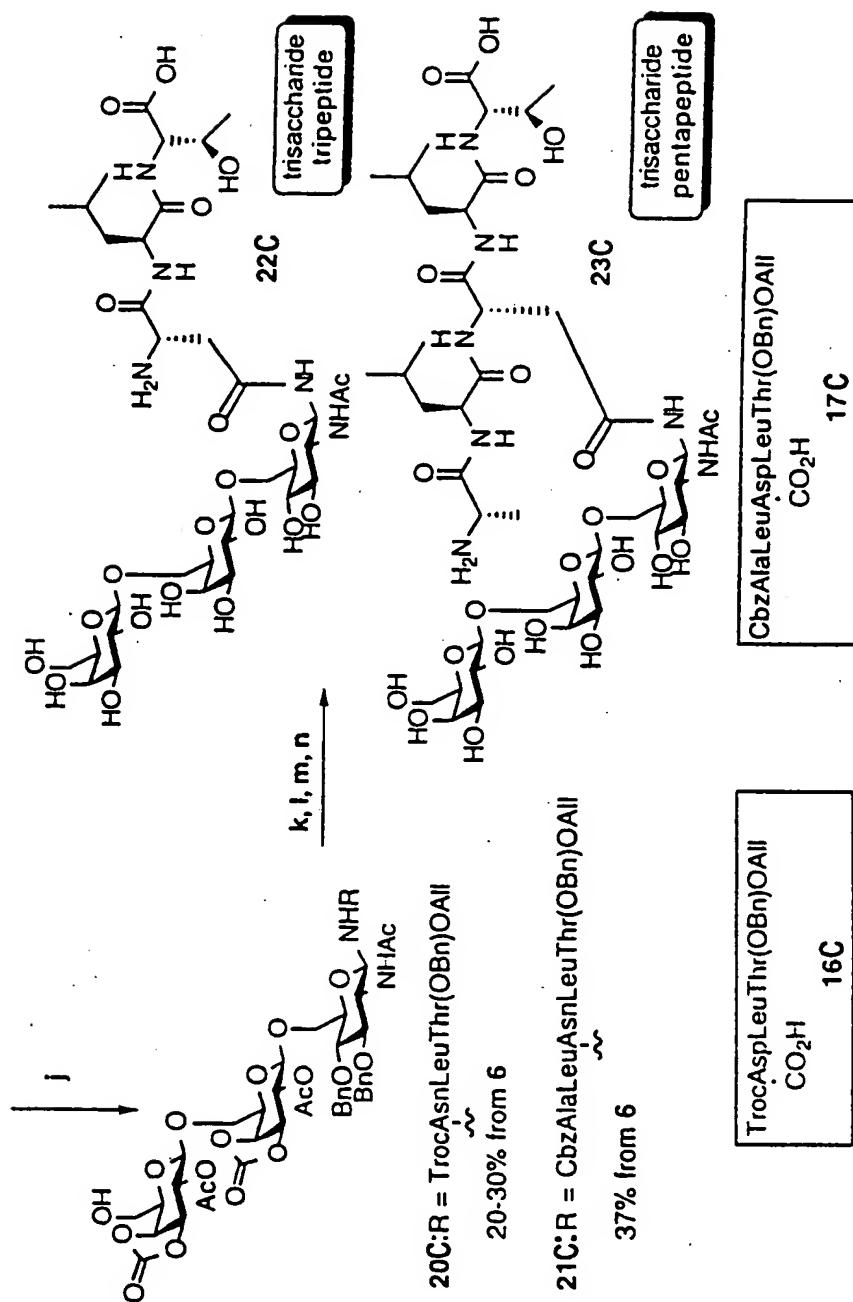
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FIGURE 15A



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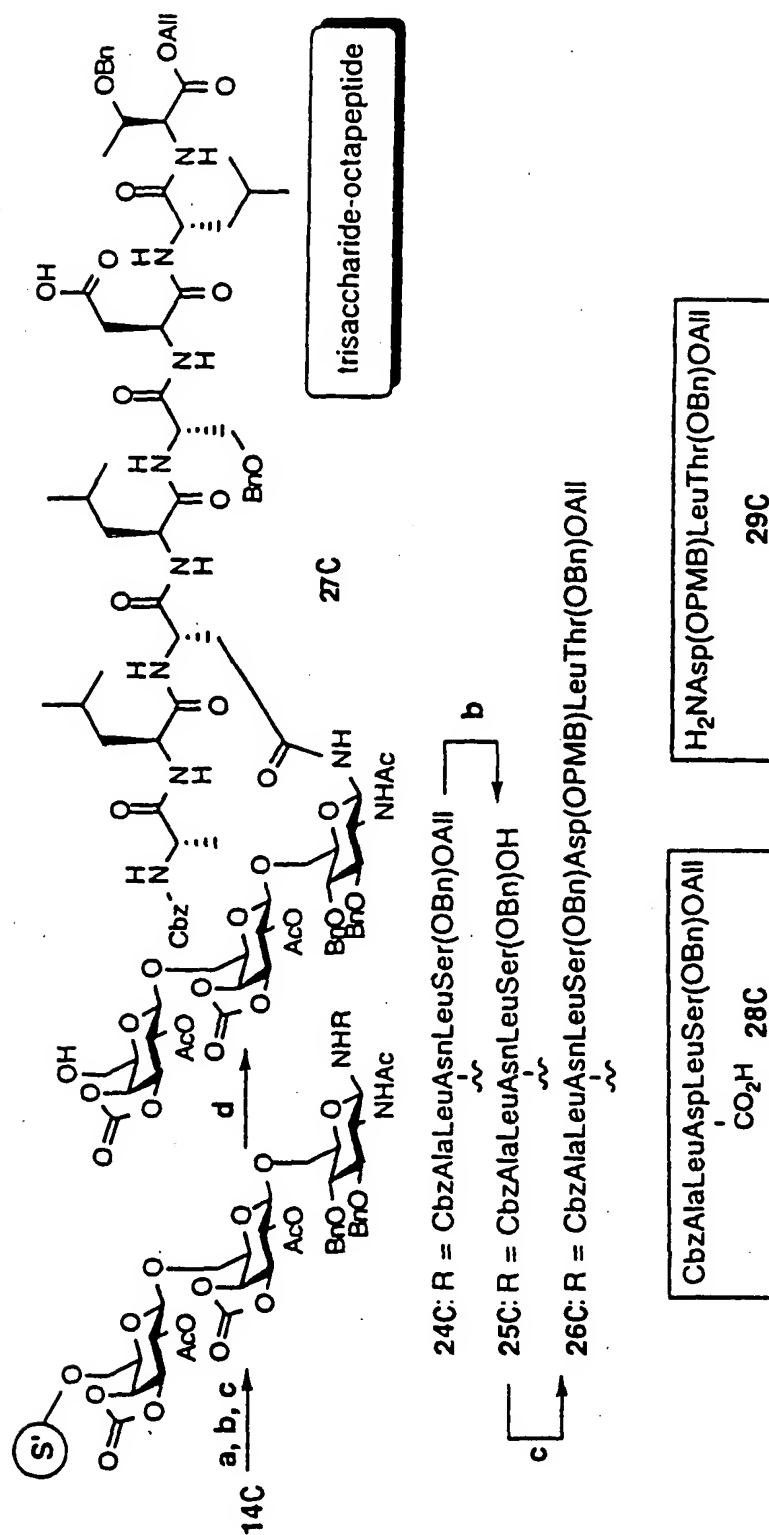
FIGURE 15B



Scheme III:

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FIGURE 16



Scheme IV:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/10229**A. CLASSIFICATION OF SUBJECT MATTER**IPC(6) : A61K 38/00; C07K 9/00
US CL : 530/322; 536/17.2, 17.5, 17.9, 18.2

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 530/322; 536/17.2, 17.5, 17.9, 18.2

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS: search terms: glycopeptide, glycoprotein, halosulfonamid####, azide, acylat###

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 5,451,570 (NADKARNI ET AL.) 19 September 1995.	1-20
A	US, A, 5,302,582 (VERTESY ET AL.) 12 April 1994.	1-20
A	US, A, 5,198,418 (MALABARBA ET AL.) 30 March 1993.	1-20
A	US, A, 5,185,320 (TRANI ET AL.) 09 February 1993.	1-20

☐ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
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E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*Z*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
16 SEPTEMBER 1996	04 OCT 1996

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